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FACTORS INFLUENCING THE SEVERITY OF CEREAL ANTHRACNOSE IN ALBERTA

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The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Factors influencing the severity of cereal anthracnose in Alberta", submitted by Donald E. Harder in partial fulfilment of the requirements for the degree of Master Of Science.

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ABSTRACT

Anthracnose of cereals and grasses, caused by Colletotrichum graminicolum, is becoming an increasingly important disease in Alberta. It was found to be the most prevalent in north-central Alberta, while only trace amounts were found in portions of the province south of Drumheller. Oats was most frequently affected.

Anthracnose is not associated with any general soil type, although the disease is often more severe on crops grown on degraded soils. Crops grown on soils with very high organic matter contents are nearly disease-free. Certain factors of the soil such as mineral salt concentration and hydrogen-ion concentration could not be correlated with disease incidence. There appeared to be a relationship between the amount of soil organic matter and the occurrence of anthracnose.

C. graminicolum responded favorably to a concentration of 0.175 grams of nitrogen per litre of medium, which is lower than that found for two other common soil fungi. The optimum temperature for growth and germination was high at 28°-30°C, while the optimum temperature for sporulation was much lower at 15°C. A high temperature of 28°-30°C favored disease initiation and development. A pH value of 7.5 was the most favorable for the growth of C. graminicolum, while there were two pH optima for each of sporulation and germination, those being 6.0 and 8.5, and 5.0 and 8.0 respectively.

C. graminicolum was found to be a poor competitor in the soil. Germination of conidia of the fungus was completely inhibited by six different soils. Autoclaved soils did not show the inhibitory effect.

Organisms antagonistic to C. graminicolum were isolated from 24 different soils. Soils with high organic matter contents contained more antagonistic organisms than did degraded soils. The majority of the antagonists were Streptomyces spp. C. graminicolum was considerably more sensitive to the Streptomyces spp. than were two other common soil fungi. Organisms antagonistic to C. graminicolum on agar cultures were also antagonistic in the soil. Aqueous, autoclaved soil extracts of 7 different soils produced both inhibition and stimulation to the growth of the fungus. However, it was concluded that the major fungistatic effect of the soil was biological in nature.

The main factors that influence the severity of cereal anthracnose are: the low nitrogen requirements of C. graminicolum; the low temperature for optimum sporulation and high temperature for disease development; and the high sensitivity of the fungus to certain microbial antagonists.

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INTRODUCTION

Anthracnose, caused by Colletotrichum graminicolum (Ces.) Wils., is distributed widely on cereals and grasses. Its occurrence in Alberta was first reported in 1933 by Sanford (41). Two years later he described its effects on oats, and stated that, under certain conditions, this crop could be damaged severely by the root-rot phase of the disease. Sanford also indicated that anthracnose was associated with degraded soils. A thorough survey of the distribution of cereal anthracnose in Alberta, however, was not conducted at that time. Since 1935 this disease has received very little attention in Canada.

Cereal anthracnose is difficult to diagnose during the major period of plant growth. This could possibly be one reason why it has been considered a disease of minor importance. General reduction in vigor of plant development, weakened, thin stems, and premature ripening are gross symptoms. These conditions can be easily mistaken for those caused by low or unbalanced soil fertility or by drought. Signs of the disease, consisting of black, setose acervuli appear at plant maturity. It is at this stage that the cause of the disease can be easily diagnosed.

Since 1961 plant pathologists at the University of Alberta have been observing cereal anthracnose more closely. The extent of its distribution and the severity of its effects, particularly on some cereal crops, has aroused renewed interest in the disease. Casual observation has also indicated that the disease might be favored by certain types of soil or soil conditions.

The first objective of the present investigation was to survey the grain areas of Alberta to determine the distribution and severity

of anthracnose on wheat, oats, barley, and rye. This survey was to take careful account of any association that the incidence of anthracnose may have with soil type and condition.

The second purpose of this study was to determine some specific factors of climate or soil that would account for the pattern of distribution. Certain physiological properties of the pathogen, including its ability to compete with other microorganisms in the soil, were investigated. Another phase of the study was a preliminary investigation of some of the factors influencing disease initiation and development. An attempt is made to relate results of laboratory and greenhouse studies to the distribution of cereal anthracnose in Alberta.

GENERAL LITERATURE REVIEW

Causal Fungus

Dicladium graminicolum Ces. was the name first given to the causal organism of anthracnose of cereals and grasses by Cesati in 1852 (4). Manns, in the paper of 1909 by Selby and Manns (43), gave the name Colletot richum cereale to the anthracnose fungus. This has been one of the more commonly used names to describe the fungus. Wilson (51) named the fungus Colletotrichum graminicolum, and listed ten synonyms. Böning and Wallner (5) have listed eight other names they consider to be synonymous with C. graminicolum. Since 1914 the latter name has been used almost exclusively. Arx (2) considered C. graminicolum to be the imperfect stage of Glomerella tucumanensis.

The acervuli are superficial, circular to oval, with dark mycelium forming the basal stroma. The black to dark-brown setae form through or surrounding the conidial-forming stroma of the mycelium. Setae are septate and tapering at the apex. Conidia are spindle-shaped, slightly curved, hyaline, and one-celled (10).

Host Range And Distribution

The first account of cereal anthracnose was given by Selby and Manns in 1909 (43). Rye was the principal crop affected, but the fungus was also found on wheat, oats, timothy, red-top, Kentucky blue-grass, orchard grass, and chess. Since that time many other hosts have been found. Sprague (44) listed 113 species of graminaceous plants from which C. graminicolum has been isolated. All of our common cereal crops were included. The fungus also has been isolated from

legume plants. Garren (15) found small amounts of C. graminicolum present on white clover at high temperatures. Tiffany and Gilman (47) isolated the fungus from red clover and lucerne.

Colletotrichum graminicolum has been found in nearly all parts of the world. It was first reported in the United States by Selby and Manns in 1909. In Canada the disease was first discovered on wheat by Gussow in 1917 (42). Since then anthracnose has been reported from China (1926), Burma (1934), India (1936), Germany (1936), Kenya (1936), Australia (1937), Italy (1939), Phi lipines (1941), Great Britain, France, New Zealand, Peru, and five African countries.

Symptoms And Economic Importance

The symptoms of cereal anthracnose are not distinct. Sanford (42) credited the major portion of the disease on oats as damage to the seminal roots with subsequent retardation of seedling growth. The plants appeared to recover after the formation of secondary roots. Dickson (12) described the gross symptoms as reduction in vigor with premature ripening or dying. In many cases, however, the symptoms differed depending on the crop. Williams and Willis (50) found C. graminicolum causing stock rot, leaf blight, and ear rot of corn. Root rot and seedling blight tests on corn, wheat, and oats were positive. However, foliage blight tests on the latter two crops were negative. Koehler (25) described leaf spotting, root rot, and stock rot of broomcorn. Two forms of the disease have been described for sorghum by Lebeau and Stokes (27). Leaf spot and stock rot of sorghum were described as

anthracnose and red rot respectively. Both were caused by the same organism. The leaves were affected at any stage during growth while the stocks were affected only during maturation of the crop. Lodging of the crop was frequently encountered as a result of the weakening of lower nodes.

Anthracnose can be readily diagnosed on a mature plant. At this time numerous black, elliptical acervuli appear at the crown region and around the lower nodes. Occasionally they may be found on the head. Under adequate moisture conditions the setae as well as the curved, pointed conidia characteristic of Colletotrichum appear in the acervuli.

Anthracnose of cereal crops may be more important than is generally realized. Barker and Neale (3) reported that in some areas of Mississippi anthracnose of rye was so severe that cultivation of the crop was discontinued. Rosen (40) stated that it was one of the major diseases of oats in Arkansas in 1949. Bruehl and Dickson (8) considered anthracnose to be a disease of increasing importance.

Generally, the sorghums appear to be the most frequently as well as most severely damaged crops (12). The extent of damage on crops, however, appears to vary with the locality. In the states of Mississippi and Alabama rye is the most seriously affected, while in Alberta the disease has been noted more often on oats (42).

Means of Dispersal

The anthracnose fungus may be perpetuated in several ways. Infected

plant residues are probably the most important single source of inoculum. Bell (4) noted that farmers in Ohio reported low yields from wheat following wheat. C. graminicolum was associated with the reduction. Lebeau and Stokes (27) found that the fungus survived on field residues of sorghum, broomcorn, sudan grass, Johnson grass, Erianthus sp., and some weeds. Sanford (42) noted that the sclerotia-like bodies on infected oat residues remained viable at least until May of the following year.

The seed also appears to be important in carrying over the anthracnose fungus. Luthra (34) found that sorghum seed was infected. Leukel, et al (28) outlined methods for the treatment of sorghum seeds to control anthracnose. Castellani (9) advised the treatment of seeds to reduce anthracnose on Ergrostis in East Africa.

The spread of anthracnose on cereals has been associated with an insect, Calendia parvula, by Hanson, et al (20). It was noted that the insect attacks lower internodes and roots, allowing the entry of pathogens. Colletotrichum was one of several pathogens isolated from plants infected in this way. The pathogens were carried both on and in the bodies of the insects.

Sorghum plants in Burma were infected both from the soil and aerially (46). The survival ability of C. graminicolum in various habitats, especially soil, needs to be investigated further.

DISTRIBUTION OF CEREAL ANTHRACNOSE IN ALBERTA

Survey Procedure

Six survey trips were made in the province of Alberta to determine the extent and severity of anthracnose on cereal crops. Their dates and general locations were as follows:

1. May 7-8 1963 - north and east of Edmonton
2. May 15 " - west of Edmonton (Breton district)
3. July 5 " - west of Edmonton (Breton district)
4. August 12-13 " - Lethbridge district
5. September 16-21 " - most of the farming areas in Alberta south of Edmonton
6. September 27 " - northwest of Edmonton (Barrhead district)

Side roads were used in the survey of farming districts. Fields of wheat, oats, barley, and rye were inspected at intervals of approximately five to seven miles. Stubble fields were ideal to determine the prevalence of anthracnose because acervuli appeared most abundantly at the lower nodes. Disease ratings of 0 to 6, based on the percentage of plants infected and the density of acervuli, were made for each field. Soil characteristics such as color and texture were noted and soil samples were collected.

Survey Results

Four hundred and forty fields were checked during the survey. The disease was found on all of the four major cereal crops of wheat, oats, barley, and rye. Oats appeared to be affected the most severely. Symptoms and signs of anthracnose on oats are shown in Figs. 1 to 4.

The survey of July 5 into the Breton district was made to determine the incidence of anthracnose on young plants. Plants which showed possible symptoms were returned to the laboratory. C. graminicolum was not isolated from any of these plants.

The distribution of cereal anthracnose in Alberta is shown on the map (Fig.5). It shows the approximate areas covered by the survey as well as the distribution of the disease. The incidence of anthracnose is shown by the relative density of the black dots. Areas not showing dots were not surveyed. The map also shows the major soil zones of Alberta; Brown (Medicine Hat), Dark Brown (Lethbridge), Thin Black (Stettler, Calgary), Black (Red Deer, Edmonton), and Grey Wooded (areas north and west of the black zone).

Anthracnose is most prevalent in north-central Alberta. Only trace amounts of the disease are found in the portion of the province south of Drumheller. Areas near the foothills and one small region near Fort McLeod are exceptions. Black, sclerotia-like bodies which appeared to be those of Colletotrichum were found on one-year old straw material, but rarely on stubble of the current year in the southern areas.

Anthracnose is often severe in local ized areas. These regions are usually composed of degraded type soils. It was also noted that in those fields where disease incidence was very high, the soil appeared to be low in organic matter. This relationship, however, was not consistent.

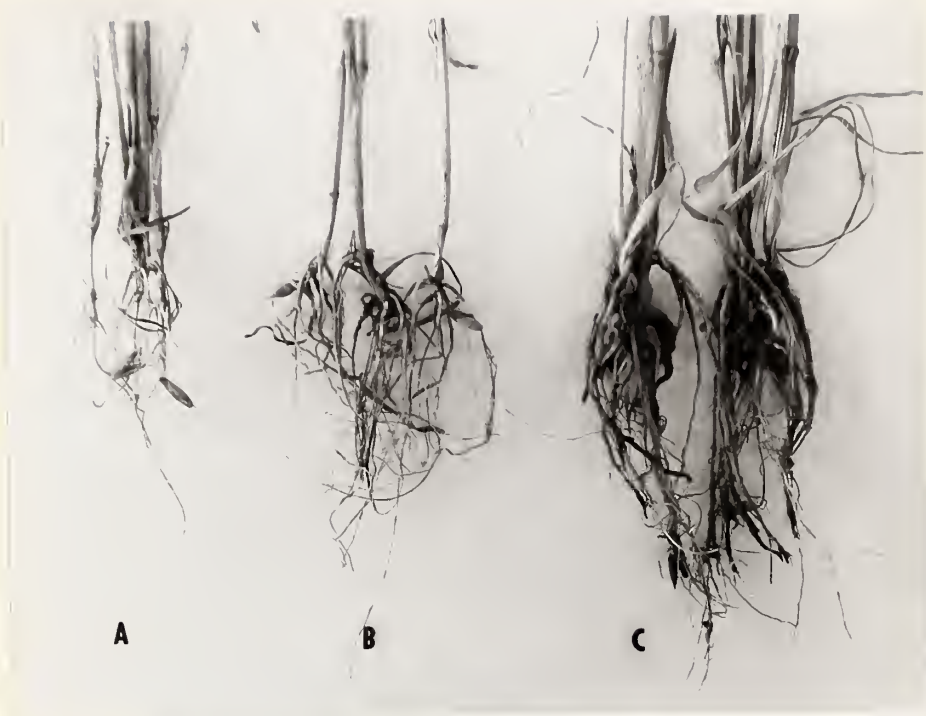

Fig. 1. Reduction of root size in oats caused by Colletotrichum graminicolum. (A) severe, (B) moderate, and (C) healthy.

Fig. 2. Stunting effect on oats caused by Colletotrichum graminicolum. Left - disease free; right - diseased.

Fig. 3. Lodging of oats caused by Colletotrichum graminicolum.

Fig. 4. Elliptical, black acervuli of Colletotrichum graminicolum that appear at maturity on lower portions of the stem. Heaviest concentration is around the nodes.

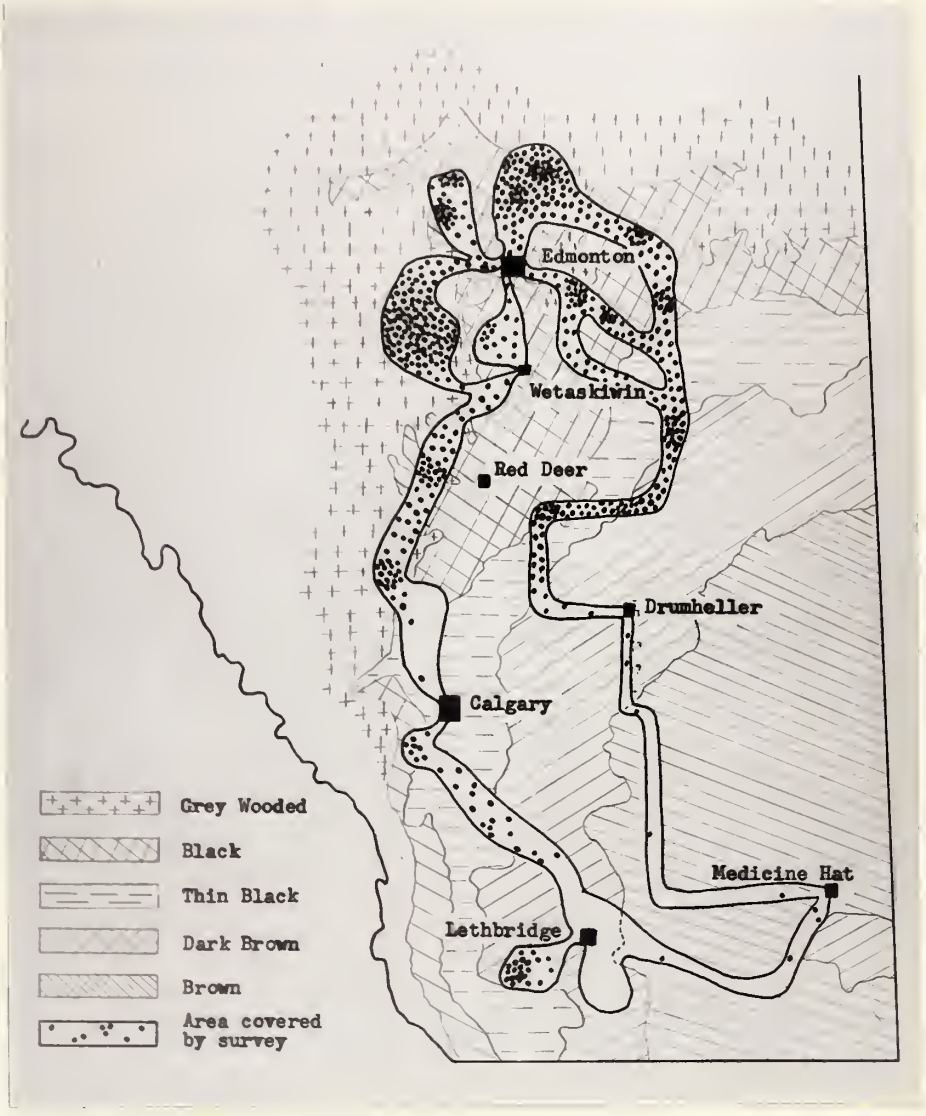

Fig. 5. Distribution of cereal anthracnose in Alberta. The incidence of disease is indicated by the relative density of the black dots.

Analysis of Soils

Methods And Materials

Soil samples were selected to represent variation in disease incidence on different soil types. Twenty-two samples were analyzed by the Provincial Soil And Feed Testing Laboratory, University Of Alberta, for nitrate nitrogen, phosphorus, potassium, soil reaction, conductivity, sulfate, and free lime.

The total organic matter content of twenty-six samples was determined by a rapid wet oxidation technique as described by Walkely (49). This procedure involved charring the soil organic matter with concentrated sulfuric acid, oxidation of the remaining carbon with potassium dichromate, and finally, titration of un-used potassium dichromate with ferrous sulfate. The results are expressed as the percent(ww) of organic matter in the soil. The organic matter is estimated as 1.72 times the determined organic carbon content, since soil organic matter is about fifty-eight percent carbon.

Experimental Results.

The results of the analysis by the Soil And Feed Testing Laboratory are shown in Table 1. No correlation could be established between the severity of disease and any of the factors for which the soil was analyzed.

The soil organic matter determinations are shown in Fig. 6. The percentages of organic matter and the disease ratings are shown on the same axis. The same figures were used to indicate the values of both organic matter and the disease rating. For example in soil sample number one, the white bar in black outline and the shaded black bar

Table 1. Analysis of 22 different soil samples representing differences in incidence of anthracnose on cereal crops.

Sample No.	Pounds Per Acre Nitrate Nitrogen (N)	Phosphorus (P)	Potassium (K)	Soil reaction pH	Conductivity mmhos.	Sulfate SO_4	Free Lime
1	20	51	100	6.3	0.8	nil	nil
2	13	80	76	6.7	0.3	"	"
3	13	46	74	6.3	0.2	"	"
4	38	nil	206	7.7	4.3	700	very high
5	13	23	144	7.3	0.8	nil	nil
6	9	6	104	7.6	0.4	"	"
7	11	16	106	6.5	0.2	"	"
8	13	28	204	6.4	0.2	"	"
9	18	26	600	7.9	0.7	Nil	high
10	18	44	396	7.6	0.5	"	nil
11	13	39	208	7.8	0.4	"	"
12	13	32	128	7.7	0.3	"	"
13	18	4	60	6.8	0.4	"	"
14	16	39	316	6.1	0.3	"	"
15	16	9	384	7.8	0.5	"	very high
16	11	73	272	7.0	0.5	"	nil
17	18	41	232	7.4	0.4	"	"
18	20	23	318	7.8	0.9	"	high
19	11	8	48	7.1	0.4	"	nil
20	7	144	208	6.9	0.3	"	"
21	11	52	74	5.5	0.3	"	"
22	18	30	48	5.4	0.3	"	"

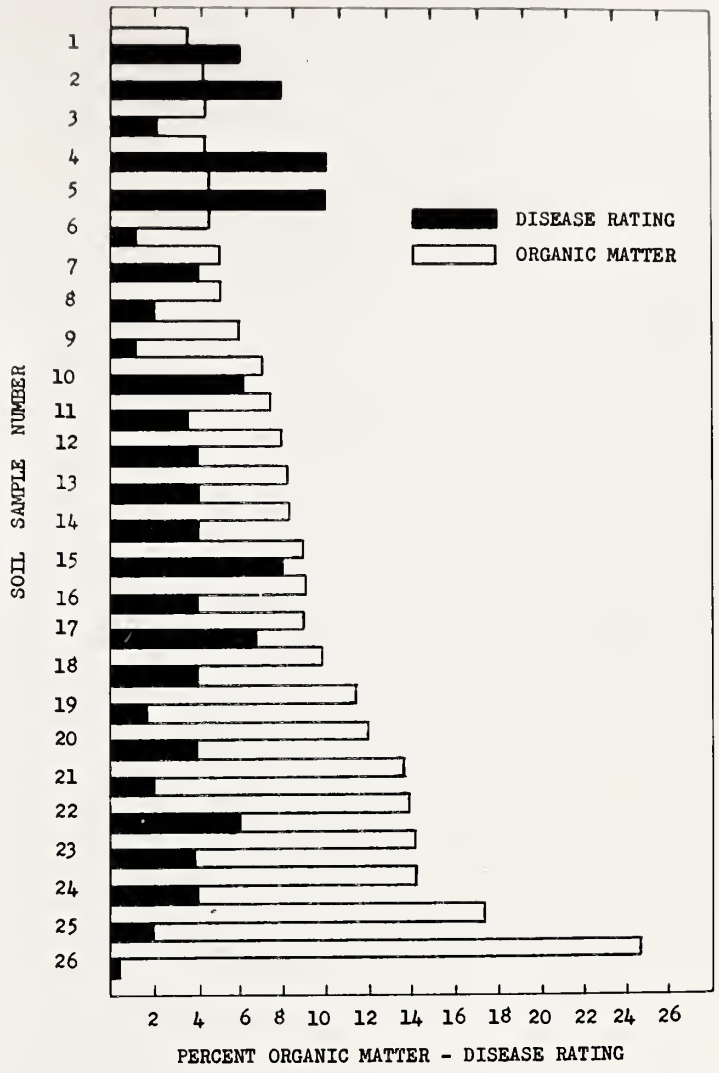

Fig. 6. Relationship between quantity of organic matter and the severity of cereal anthracnose.

indicate the organic matter and disease rating for the field from which soil sample number one was taken. There appears to be a correlation between disease severity and the quantity of organic matter in the soil. High disease incidence was generally associated with low organic matter content, and the reverse was also true.

Discussion

The organic matter content of the soil seems to be the only factor for which there is some correlation with disease severity. In this respect some interesting observations were made in the Grey Wooded soil zone. Some of the fields contained low spots which were composed of peat soils. The organic matter content was very high. The surrounding area usually consisted of a degraded grey soil with low organic matter content. Crops in the degraded portions of the field often showed a fairly high incidence of anthracnose, while there was no evidence of the disease in the peaty areas of the same field. Crops in other peat soils were also free of the disease.

Attempts to correlate disease incidence with soil factors may not be valid because the cropping history of individual fields was not known. Host specialization in C. graminicolum has been reported by Bruehl and Dickson (8), Lebeau (26), and Bell (4). Since primary infection arises from infected straw material of the previous year, continuous cropping without rotation would lead to a high inoculum potential with consequent high levels of disease. On the other hand crop rotation would be expected to keep disease levels down. However, since anthracnose appears to be more prevalent on degraded soils, it is possible that some soil factor might over-ride the effects of crop

rotation. The organic matter content of the soil may be one such factor. There appears to be some correlation between disease levels and the quantity of organic matter. The fact that anthracnose occurs in crops in the degraded portions of a field but not in the peaty spots of the same field confirms this hypothesis. Thus the effect of high organic matter in the soil may well mask the effects of cropping or soil management practices.

PHYSIOLOGICAL PROPERTIES OF *COLLETOTRICHUM GRAMINICOLUM*

A relationship between the severity of anthracnose and the condition of the soil may exist. Nitrogen is a major factor in soil fertility as well as in the ability of fungi to grow in the soil (24). The nitrogen requirement of *Colletotrichum graminicolum* was therefore investigated.

Temperature and reaction are important factors of the soil environment. The investigation of the physiological properties of *C. graminicolum* included a study of the effect of temperature on growth, sporulation, and germination, and the effect of hydrogen-ion concentration on growth, sporulation, and germination.

Literature Review

Nitrogen nutrition

The nitrogen requirements of fungi differ considerably. Fungi have been classified on their ability or inability to utilize certain forms of nitrogen. Sources of nitrogen usually include atmospheric nitrogen, nitrate nitrogen, nitrite nitrogen, ammonium nitrogen, and organic nitrogen (29). The ability of a fungus to utilize a nitrogen source, especially ammonium nitrogen, may not be absolute. Many workers (reviewed by Cochrane (11)), have noted that the growth of fungi caused a sharp drop in the pH of media containing ammonium salts. Cochrane (11) suggested that the inability to utilize ammonium nitrogen may reflect only the inability to grow at lower pH values. There are possible errors in claims for the unsuitability of certain nitrogen sources. For example, some amino acids reduce the inhibitory effects of heavy

metals present in the basal medium; or, certain "essential" sources of organic nitrogen may be effective due to their vitamin content (Cochrane (11), pp. 246).

Few references concerning the nitrogen requirements of Colletotrichum are present in the literature. Ali (1) found L(+) asparagine and yeast to be good sources of nitrogen for an alfalfa isolate of C. graminicolum, and L(-) arginine, ammonium nitrate, and potassium nitrate as good sources of nitrogen for a wheat isolate of the same fungus.

The concentration of nitrogen in the medium presents more difficulties. Cochrane (11) maintains that there is no optimum amount of nitrogen for a culture. The presence of many factors, especially carbon supply, may alter the apparent optimum concentration of the nitrogen source. There is little experimental evidence as to why this is so. However, concentration of nitrogen has an effect on growth of fungi. Ali (1) found that the dry weight of a wheat isolate of C. graminicolum increased in a medium with a low concentration of nitrate nitrogen. The dry weights of orchard grass and alfalfa isolates were unaffected by the nitrate concentration.

Temperature

Temperature affects all activities of organisms. Growth curves are characterized by a linear increase in growth with increase in temperature, an optimum range, and a decrease in growth as the temperature becomes too high. The temperature optima for growth may also be influenced by other factors. Nitrogen supply, biosynthesis of certain compounds necessary for growth, and hydrogen-ion concentration have been shown to affect the temperature optimum (11). However, if conditions

of time, medium, and other external ones are specified, temperature optima for growth are generally held to be valid. The temperature of most plant pathogenic fungi fall in the 20°- 30°C range, with many of these being between 26° and 30°C (11). Ali (1) found two temperature optima for the growth of three isolates of Colletotrichum graminicolum. There were two minima at 10° and 25°C, and two optima at 20° and 30°C.

The temperature most favorable for sporulation is often different from that required for growth. It is commonly agreed that the temperature limits for sporulation are narrower than those for growth (11). The optimum temperature for sporulation may be near the optimum for growth, as in Piricularia oryzae (11); above the growth optimum as in Sphaerotheca pannosa (11); or lower, as has been found for Colletotrichum lindemuthianum (35). Sporulation of C. lindemuthianum occurred best between 15° and 20°C, which is a full 10° below the optimum temperature for growth.

The influence of temperature on germination of spores is generally similar to that on mycelial growth. (6, 11). Temperature affects the rate of germination, the percentage of germination, and the growth rate of the germ tube. All three factors have been used as measurements for germination. The optimum temperatures for spore germination vary considerably. Optima range from a low of 9°C in Peronospora effusa to a high of 43°- 45°C in Rhizopus chinensis (11).

Hydrogen-ion concentration

The responses of fungi to hydrogen-ion concentration are varied. Maximum, minimum, as well as optimum pH values have been determined for a large number of fungi. Most plant pathogenic fungi grow best in media

with an initial pH of 5.0 to 6.5 (29). The pH range for good growth may be quite wide, as exemplified by Colletotrichum hibisci, whose optimum ranges from 3.5 to 8.0 (48). Ali (1) found that the dry weight of Colletotrichum graminicolum increased in pH range of 4.0 to 6.0, above which the dry weights decreased. As in the effects of other external factors on the growth of fungi, the pH growth curve must be qualified. The pH effects are modified by such factors as temperature and chemicals in the medium. Media must be well buffered, as the growth of fungi may depress their pH values (29, 11).

The pH optima for sporulation of fungi are generally in a narrower range than those for growth. Generally, neutral or slightly alkaline reactions are more favorable for sporulation than are acidic ones (39, 45).

Spore germination is also affected by hydrogen-ion concentration. However, it is not usually the limiting factor in germination. There appears to be some discrepancy in the pH ranges for germination. Cochrane (11) stated that the pH range for germination is usually narrower than that for growth, while Lilly and Barnett (29) indicated a wide range for germination. Webb (in Cochrane (11)) determined pH optima for germination of a large number of fungi. The optimum for most species was pH 3.0 to 4.0. Colletotrichum gossypii germinated best at a slightly alkaline pH. Cochrane (11) indicated that most fungi germinated best at pH 4.5 to 6.5, with limits at about pH 3.0 and 8.0. Again, complicating factors must be considered. Strains of a given species differ in response; the type of buffer used may have an effect; nutrient materials modify pH response; and the previous history of spore populations should be considered.

Nitrogen Requirements of Colletotrichum graminicolum

Methods And Materials

A single spore culture of Colletotrichum graminicolum was isolated from infected oat straw obtained from the Lethbridge area. The oat isolate was used for all studies on C. graminicolum. All future reference to the fungus will be given as C. graminicolum.

Laboratory stock cultures of Fusarium culmorum (W.G. Smith) Sacc. and Rhizoctonia solani Kuehn were used for comparison with C. graminicolum.

The growth responses of C. graminicolum, F. culmorum, and R. solani to various nitrogen concentrations were determined by obtaining oven-dry weights of mycelium produced on liquid shake cultures. Fifty ml. of medium were used in two hundred ml. Erlenmeyer flasks.

A general purpose synthetic medium was used. This medium will be referred to as "basal" in further discussion. The composition of the medium, without nitrogen, is as follows:

sucrose	38.0 grams
KH_2PO_4	0.3 grams
K_2HPO_4	1.2 grams
$MgSO_4 \cdot 7H_2O$	0.3 grams
$FeCl_3 \cdot 6H_2O$	1.0 ppm.
$ZnSO_4 \cdot 7H_2O$	1.0 ppm.
$CuSO_4 \cdot 5H_2O$	1.0 ppm.
$MnSO_4 \cdot 5H_2O$	1.0 ppm.
de-mineralized H_2O	1000 ml.

Three different nitrogen sources were used. These were: ammonium phosphate, ammonium nitrate, and potassium nitrate. These compounds were added to the "basal" medium to give a range of 0.0 to 0.8 grams of nitrogen per litre.

Three methods of inoculating the flasks containing the medium were investigated in order to obtain the most consistent results within replications. The methods are outlined as follows.

Method A

Petri dishes containing potato-sucrose agar were inoculated with C. graminicolum, and incubated at 24°C. The tip of a six-inch glass rod was bent and flattened to form a shoe. After several days of growth a four mm. disc of C. graminicolum was as eptically cut from the advancing edge of the colony, and placed on the sterile glass shoe. The glass shoe bearing the fungus disc was placed in an Erlenmeyer flask so that its surface came in contact with the medium. The fungus was incubated at 24°C for six days.

Method B

A disc of the fungus was cut as described above, and placed in fifty ml. of medium in a two hundred ml. Erlenmeyer flask. The fungus was incubated at 24°C on a shaker.

Method C

A heavy suspension of a mixture of mycelium and conidia of C. graminicolum was obtained by homogenizing the fungus in water in a Waring blendor. Two ml. of the suspension were pipetted into 50 ml.

of medium contained in 200 ml. Erlenmeyer flasks, and incubated at 24°C on a shaker.

In the above methods, growth was measured when the mycelium appeared to have filled the medium. The fungus was filtered from the medium, rinsed, and dried at 90°C for twenty-four hours. Filter papers were oven-dried and pre-weighed.

Method C. gave the most consistent results and, therefore, was used in all subsequent determinations. Rhizoctonia solani and Fusarium culmorum were treated in a similar manner.

All glassware used in these and subsequent tests was immersed in a concentrated chromate-sulfuric acid solution for twelve hours. It was rinsed with tap water, then with distilled water, and finally allowed to soak in distilled water for twenty-four hours.

The optimum nitrogen concentration determinations were carried out in two steps. Initially a wide range of nitrogen concentrations was used. The concentrations were 0.0, 0.05, 0.1, 0.2, 0.4, and 0.8 grams of nitrogen per litre. Each treatment was replicated four times.

The second step involved a narrower range of nitrogen concentrations, which were 0.0, 0.05, 0.075, 0.10, 0.125, 0.150, 0.175, 0.20, and 0.30 grams of nitrogen per litre. The temperature was held constant at 75°F. Each treatment was replicated ten times.

Experimental Results

Nitrogen source

The dry weights of mycelium of C. graminicolum resulting from

growth in ammonium nitrate, ammonium phosphate, and potassium nitrate are shown graphically in Fig. 7. Greater maximum growth occurred in the medium containing ammonium nitrate than either ammonium phosphate or potassium nitrate.

Determinations of pH of the media were made before and after growth. The initial pH values of all three media at all concentrations of nitrogen ranged from 6.5 to 7.2. In the medium containing ammonium phosphate the pH values dropped progressively to 2.5 as the nitrogen concentration increased. In the media containing ammonium nitrate or potassium nitrate the pH values dropped to 3.0 as the nitrogen concentration increased to 0.40 grams per litre, and rose to 3.6 at 0.80 grams nitrogen per litre. The drop in pH was not as rapid in ammonium nitrate as in the other two nitrogen sources. Ammonium nitrate was used as the nitrogen source in all subsequent determinations.

Nitrogen concentration

The dry weights of mycelium of C. graminicolum, Rhizoctonia solani, and Fusarium culmorum over the wide range of nitrogen concentrations are shown graphically in Fig. 8. The increase in dry weight of C. graminicolum at the low nitrogen concentration is greater than the increase of R. solani or F. culmorum. The peak of the growth curve of C. graminicolum is reached sooner than for the other two fungi. Also, the dry weight of mycelium of C. graminicolum drops more rapidly after the peak is reached than that of R. solani or F.culmorum.

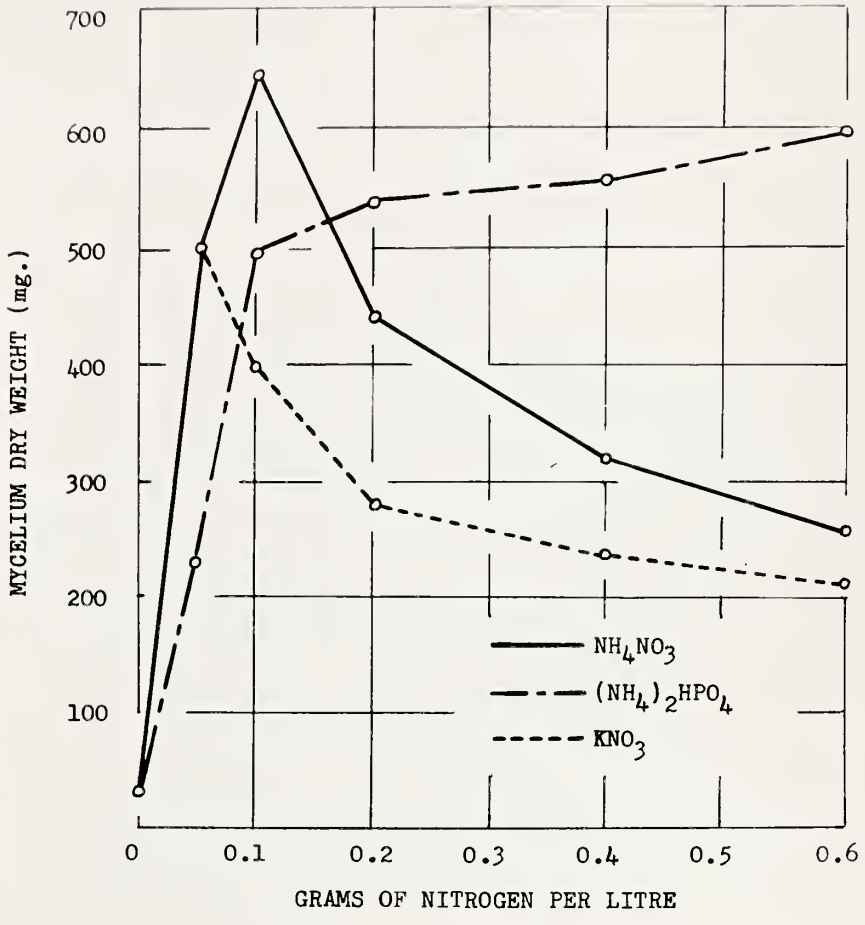

Fig. 7. Dry weight of mycelium of Colletotrichum graminicolum obtained in different concentrations of ammonium nitrate, ammonium phosphate, and potassium nitrate.

The results of the second nitrogen concentration determination, made over the narrower range of concentrations, are shown graphically in Fig. 9. Again, the increase in dry weight of C. graminicolum, especially at the low nitrogen concentrations, is more rapid than the increase in either R. solani or F. culmorum. The peak of the growth curve for C. graminicolum is reached at 0.175 grams of nitrogen per litre, while the peaks for the other two fungi are not reached at 0.275 grams of nitrogen per litre.

Discussion

Ammonium nitrate appears to have some advantages as a nitrogen source. The two sources of nitrogen in the compound has led many workers to use it in their media. The effect of ammonium ion on pH has been noted earlier. It also has been noted that ammonium nitrate or mixed nitrogen sources in the media help to stabilize pH if the fungus is able to utilize both ammonium and nitrate nitrogen (11). The results in Fig. 7 indicate that the isolate of C. graminicolum utilizes both ammonium and nitrate nitrogen equally well. Also, there is a slower depression in pH with ammonium nitrate than with the other two nitrogen sources. The advantages of using ammonium nitrate in the medium for the growth of C. graminicolum are evident.

The optimum concentration of nitrogen for the growth of a fungus may vary. However, it appears that a constant optimum concentration can be obtained if the conditions are specified. The optimum nitrogen concentration for C. graminicolum has significance when compared with the "optimum" concentrations for R. solani and F. culmorum under the

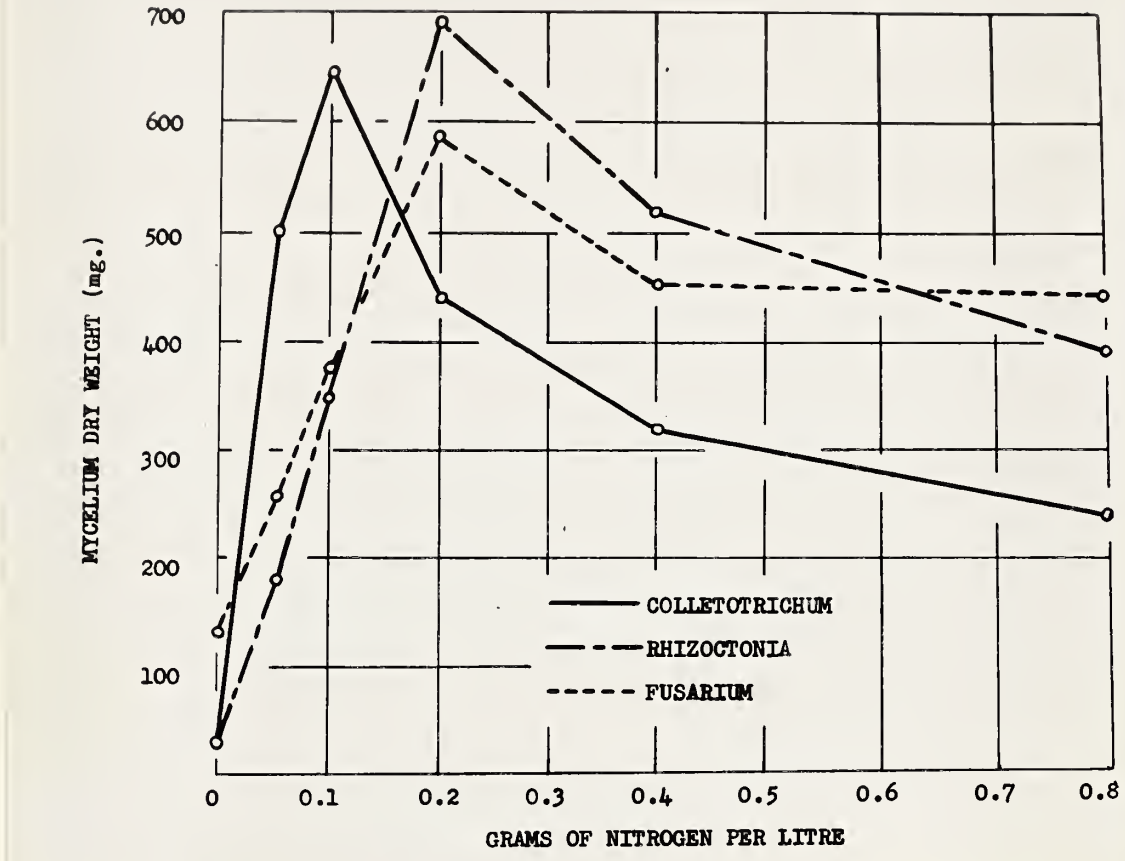

Fig. 8. Dry weights of mycelium of Colletotrichum graminicolum, Rhizoctonia solani, and Fusarium culmorum obtained in different concentrations of ammonium nitrate. The wide range of nitrogen concentrations was used.

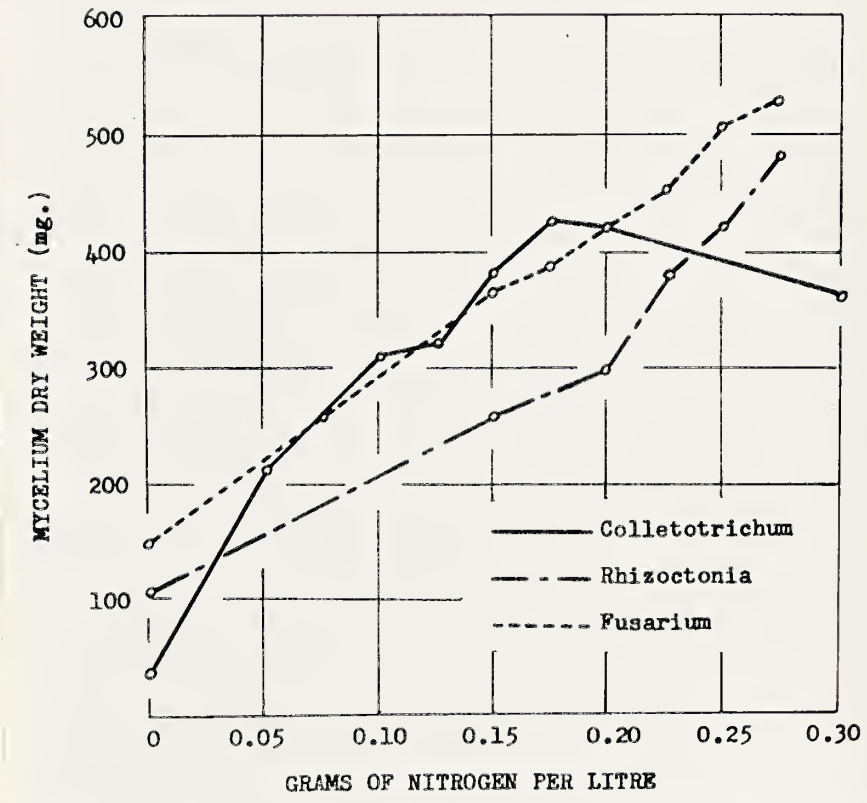

Fig. 9. Dry weights of mycelium of Colletotrichum graminicolum, Rhizoctonia solani, and Fusarium culmorum obtained in different concentrations of NH_4NO_3. The narrow range of nitrogen concentrations was used.

same conditions. The sharper increase in growth at the lower concentrations as well as the peak of growth at a lower concentration for C. graminicolum indicate its relatively lower nitrogen requirements.

The results obtained correspond with those of Ali (1), who found that the wheat isolate of C. graminicolum grew well at lower nitrogen concentrations. The implications of the low nitrogen requirement will be discussed later.

Effects of Temperature

Methods and Materials

Mycelial growth

The effect of temperature on C. graminicolum was determined by measuring radial growth of the fungus. Three media were used in the test. These were the "basal" synthetic medium with 0.15 grams of ammonium nitrate per litre, potato-sucrose agar, and malt-yeast agar. The latter two media were made up as follows:

Potato-sucrose agar:

potatoes, peeled and cut	200 grams
sucrose	20 grams
bacto-agar	17 grams
de-mineralized H_2O	1,000 ml.

Malt-yeast agar:

malt extract	5 grams
yeast extract	5 grams
dextrose	15 grams
bacto-agar	17 grams
de-mineralized H_2O	1,000 ml.

The media were autoclaved, and 30 ml. aliquots of each medium were dispensed into sterile Petri dishes.

Inoculum was obtained by cutting 4mm. discs of mycelium from the advancing edge of a four-day old colony of C. graminicolum. The discs were placed on the centre of the medium in each Petri dish, and incubated for six days at temperatures of 10°, 15°, 20°, 24°, 26°, 28°, 30°, 35°C. Each treatment was replicated five times.

Sporulation

C. graminicolum grows well on moistened, sterilized soil which has been amended with 10 percent (ww) cornmeal. Three different soils were used as media for growth and sporulation of C. graminicolum at various temperatures. The soil types and their locations were as follows:

Black Loam	Edmonton
Grey Wooded	Breton
Sandy Brown	Athabasca

The soils were prepared by passing them through a 2 mm. sieve, adding 10 percent (ww) cornmeal, and moistening to a point just short of stickiness. Fifty grams of soil were placed in each of wide-mouth 250 ml. Erlenmeyerflasks. The soil medium was autoclaved for two hours, cooled, and inoculated with a mycelial suspension of C. graminicolum.

The inoculated soils were incubated at 10°, 15°, 20°, 25°, 30°, and 35°C. Each treatment was replicated five times.

After incubation for two weeks, mycelium was scraped from the soil, mounted on a slide in cotton blue-lacto-phenol preparation,

and examined for numbers of conidia. Ratings of 0 to 5 were used to designate the relative numbers of conidia.

Germination

Infected oat straw was the source of conidia in germination tests. It was surface sterilized for one and one-half minutes in 1:1,000 mercuric chloride, rinsed, and placed in a moist atmosphere. Sporulation occurred abundantly after three days. The straw was macerated in a Waring blendor containing distilled water. The suspension was strained through several layers of cheese-cloth to remove large straw particles. This suspension contained large numbers of conidia of C. graminicolum.

The effect of temperature on germination of conidia was determined on water agar. Petri dishes containing 1.5 percent water agar were held at the desired temperature for a time prior to spraying with a conidial suspension of C. graminicolum. The seeded Petri dishes were then placed in temperature chambers at 10°, 15°, 20°, 25°, 30°, and 35°C. Five replicates for each temperature were made. Conidia were checked periodically under the microscope for germination. The lengths of the germ tube after six hours and after twenty hours were used as a measurement of the rate of germination.

Disease Development

The effect of temperature on disease development was determined in a growth chamber and in Wisconsin temperature tanks.

A growth chamber was adjusted to simulate day and night conditions.

Daytime temperatures were held at 28°C for six hours, after which the temperature was allowed to gradually drop to 21°C. The 21°C temperature was maintained for six hours, after which the temperature again gradually rose to 28°C.

The lights were adjusted so that maximum light occurred at the high temperature, and darkness prevailed at the low temperature. Relative humidity was maintained at 65 to 70 percent.

Wisconsin temperature tanks are designed to hold soil temperatures at desired levels. The soil is contained in pots or in stone crocks. Soil temperatures were held at 13°, 21°, and 30°C to determine the effects of temperature on disease development.

Oats was seeded in soil in five-inch pots. Six pots were placed in the growth chamber, and there were two pots for each temperature in the temperature tanks.

When the oat seedlings had reached the two-leaf stage, a conidial suspension of the oat isolate of C. graminicolum was injected into the crown with a hypodermic needle. The plants were grown to maturity.

Experimental Results

Mycelial growth

The effects of temperature on mycelial growth of C. graminicolum on the three media listed are shown graphically in Fig. 10. The optimum temperature lies between 28° and 30°C. The relative rates of growth at the various temperatures is the same on all three media.

Sporulation

The effects of temperature on sporulation are shown graphically

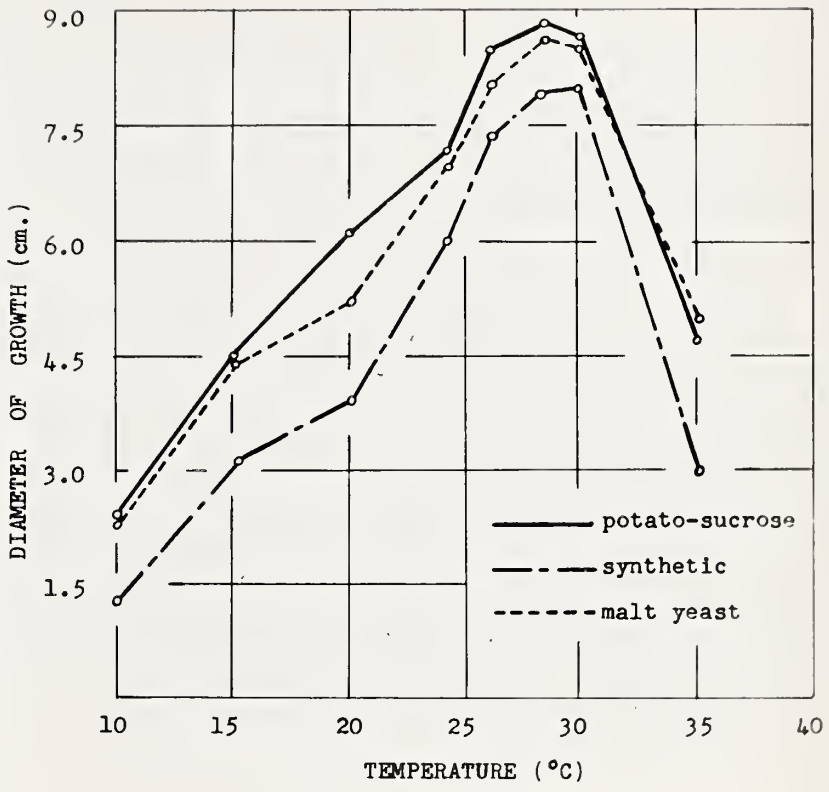

Fig. 10. Diameter of growth of Colletotrichum graminicolum on three different media at various temperatures.

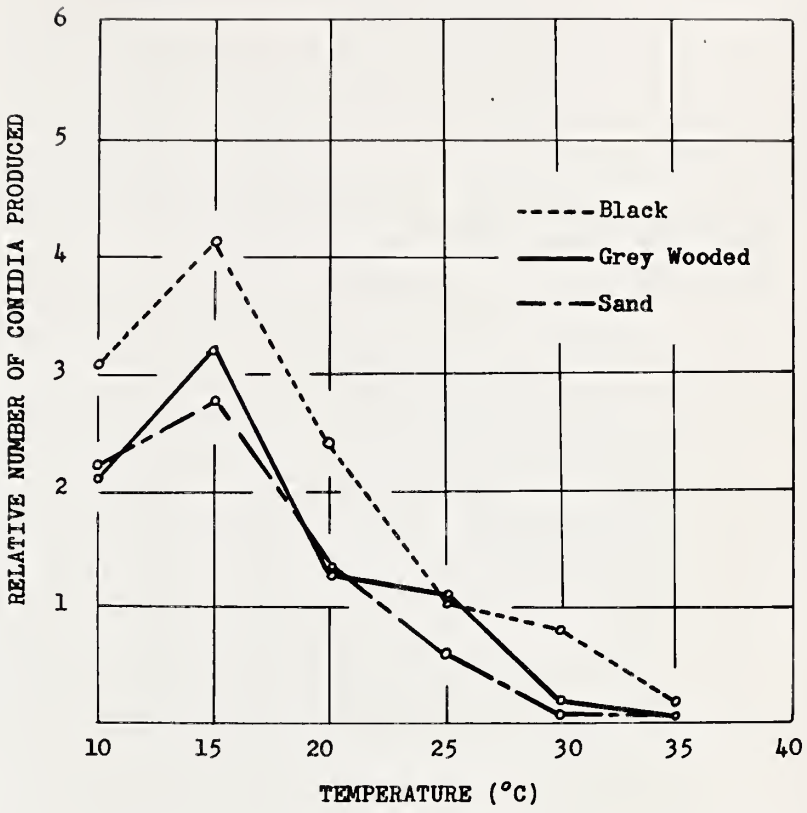

Fig. 11. Production of conidia by Colletotrichum graminicolum on three different soil types at various temperatures.

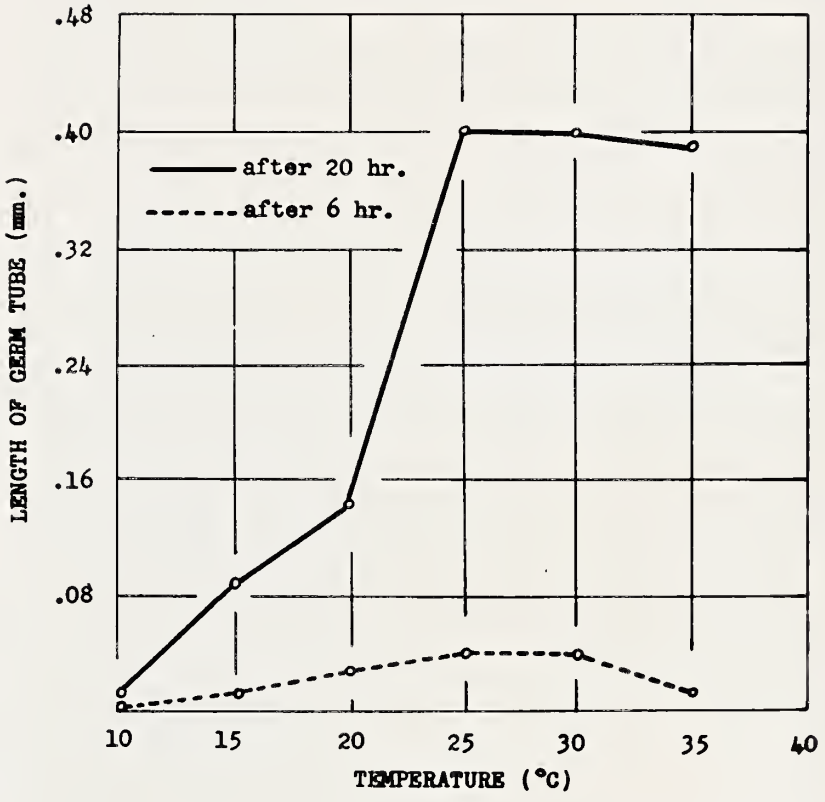

Fig. 12. Germination of conidia of Colletotrichum graminicolum, measured as length of germ tube, at various temperatures.

in Fig. 11. Maximum sporulation occurs at 15°C on each of the three soil types. The numbers of conidia produced at the various temperatures are not affected by soil type.

Germination

The rates of germ tube elongation at various temperatures are shown graphically in Fig. 12. The optimum temperature for germination ranges from 25° to 30°C. Temperatures below the optimum appear to have a greater depressing effect on germ tube elongation than temperatures above the optimum.

Disease Development

High temperatures favor disease development. Severe infection was obtained in the growth chamber. The temperature was at the upper limits of plant growth.

In the Wisconsin temperature tanks there was no disease at 13°C, slight infection at 21°C, and more severe infection at 30°C.

In the growth chamber acervuli were abundant on the lower three nodes, while in the Wisconsin temperature tanks the acervuli were present only on the sub-coronal internode.

Discussion

Cochrane (11) reported that 30°C is a maximum temperature for the optimum growth of most fungi. The average temperature was somewhat lower. The optimum temperature of 28° to 30°C for the growth of the oats isolate of C. graminicolum fell in the higher range of optimum

temperatures. The results agreed closely with those of Ali (1).

The optimum temperature for sporulation is considerably lower than that required for mycelial growth. The reduction in optimum temperature to 15°C is similar to that noted by Mathur, et al (35) for Colletotrichum lindemuthianum. The temperature optimum for sporulation does not appear to be greatly influenced by external factors as is evidenced by sporulation on the three different soil types. The soils differed greatly in their composition.

The optimum temperature for germination is close to that for mycelial growth. These results are similar to those that have been found for most fungi (29, 11).

The development of anthracnose on oats is favored by high temperature. The plants were suffering somewhat due to high temperature, yet disease development was severe. In the Wisconsin tanks the temperature of 30°C was considerably more favorable than 21°C. The location of the acervuli is further evidence that anthracnose is favored by high temperature. In the growth chamber the air temperature was equal to that of the soil temperature, while in the Wisconsin tanks the air temperature was considerably lower than the soil temperature. The appearance of the acervuli on nodes several inches above the soil surface in the growth chamber, and the restriction of the acervuli to the sub-coronal internode in the Wisconsin tanks demonstrates the importance of temperature.

Effects of Hydrogen-ion Concentration

Methods And Materials

Mycelial growth

The hydrogen-ion concentration for optimum growth was determined

by two methods. These were dry weight determinations of mycelium grown in buffered liquid medium, and measurement of radial growth on a solid agar medium.

The preparation of the basal synthetic liquid medium has been described. The sucrose concentration was reduced by one-half to keep osmotic pressure at a lower level. The directions of Gomori (18) were used to make up the buffer systems. Buffer systems over the pH range were chosen so that there was a minimum of toxicity as well as minimum nutritional effects. The directions of Gomori (18) were modified to suit microbiological work. Wherever possible, dry weights of chemicals were used instead of solutions, and the concentrations of buffering materials were reduced by three-quarters to reduce excessively high osmotic pressures.

The buffers that were used and their composition are as follows:

Theoretical pH	Acid	Salt	Actual pH
1.5	2.5 ml. 0.5 N HCl	0.280 gm. KCl	1.35
2.2	0.5 ml. 0.5 N HCl	0.280 gm. KCl	2.10
3.0	0.668 gm. $H_3C_6H_5O_7H_2O$	0.078 gm. $Na_3C_6H_5O_72H_2O$	2.80
4.0	0.475 gm. "	0.375 gm. "	4.00
5.0	0.295 gm. "	0.650 gm. "	4.95
6.0	0.138 gm. "	0.915 gm. "	5.90
6.0	0.258 gm. "	0.688 gm Na_2HPO_4	6.05
7.0	0.093 gm. "	0.935 gm. Na_2HPO_4	7.00
7.0	0.405 gm. $NaH_2PO_4 \cdot H_2O$	1.228 gm. $Na_2HPO_4 \cdot 7H_2O$	6.95
8.0	0.055 gm. $NaH_2PO_4 \cdot H_2O$	1.905 gm. $Na_2HPO_4 \cdot 7H_2O$	7.60

Theoretical pH	Acid	Salt	Actual pH
8.0	0.233 gm. H_3BO_4	0.035 gm. $Na_2B_4O_7 \cdot 10H_2O$	7.30
9.0	0.233 gm. H_3BO_4	0.443 gm. $Na_2B_4O_7 \cdot 10H_2O$	8.50
9.2	0.033 gm. Na_2CO_3	0.290 gm. $Na\ HCO_3$	9.70
9.6	0.128 gm. Na_2CO_3	0.215 gm. $NaHCO_3$	9.80
10.0	0.218 gm. Na_2CO_3	0.140 gm. $NaHCO_3$	9.95
10.7	0.358 gm. Na_2CO_3	0.033 gm. $NaHCO_3$	10.15

The weights of acid and the salt of each acid were added to 300 ml. of "basal" medium.

The pH levels at the buffer changes were over-lapped so that any differences due to nutritional effects of buffering materials could be detected. The pH values were determined before and after autoclaving.

Fifty ml. aliquots of the medium were dispensed into 200 ml. Erlenmeyer flasks and autoclaved. Each flask was inoculated with a 2 ml. homogenized suspension of C. graminicolum. Each treatment was replicated five times. The cultures were incubated on a shaker at 24°C for eight days. Harvesting of the cultures was carried out as previously described.

The "basal" synthetic medium was used in the experiments designed to measure radial growth of C. graminicolum at various hydrogen-ion concentrations. 0.150 grams of ammonium nitrate were added. The desired pH range was obtained by adjusting aliquots of the medium with HCl or KOH. 4.5 grams of Bactoagar were added to each portion.

The medium was autoclaved and dispensed into sterile Petri

dishes in 30 ml. amounts. The media in the Petri dishes were inoculated with 4 mm. discs of C. graminicolum cut from the edge of 5-day old colonies. Each treatment was replicated ten times.

The cultures were incubated at 25°C, and radial growth was measured after three, five, seven, and eight days.

Sporulation

The effect of hydrogen-ion concentration on sporulation was determined by growing C. graminicolum on soil cultures. Grey wooded soil, pH 6.5, was amended with 10 percent (ww) cornmeal. The amended soil was moistened with 0.5N HCl or 0.5N KOH to adjust the pH to values of 3.5, 5.0, 6.5, 8.5, 9.5, and 11.0. Eighty grams of soil were dispensed into 250 ml. wide-mouth Erlenmeyer flasks, and autoclaved for two hours. The final pH values of the soils were 4.4, 5.8, 6.5, 7.1, 7.2, and 8.5. The cultures were incubated at 24°C for one week, after which they were transferred to 15°C for one week. Production of conidia was determined as described in the temperature experiments.

Germination

The effect of hydrogen-ion concentration on germination of conidia was determined by seeding conidia of C. graminicolum on buffered water agar, and incubating at 30°C.

It is possible that buffering materials have an effect on germination. Two parallel series of buffers with over-lapping pH values were made to detect any possible effects.

The pH values used and their buffers are as follows:

Series (a)

pH	Acid	Salt
3.0	0.668 gm. $H_3C_6H_5O_7H_2O$	0.078 gm. $Na_3C_6H_5O_7 2H_2O$
4.0	0.475 gm. "	0.375 gm. "
5.0	0.295 gm. "	0.650 gm. "
6.0	0.138 gm. "	0.915 gm. "
7.0	0.093 gm. "	0.935 gm. Na_2HPO_4
8.0	0.233 gm. H_3BO_4	0.035 gm. $Na_2B_4O_7 10H_2O$
9.0	0.233 gm. H_3BO_4	0.423 gm. "
10.0	0.218 gm. Na_2CO_3	0.140 gm. $NaHCO_3$

Series (b)

pH	Acid	Salt
3.0	0.668 gm. $H_3C_6H_5O_7H_2O$	0.078 gm. $Na_3C_6H_5O_7 2H_2O$
4.0	0.475 gm. "	0.375 gm. "
5.0	0.295 gm. "	0.650 gm. "
6.0	0.258 gm. "	0.688 gm. Na_2HPO_4
7.0	0.405 gm. $NaH_2PO_4 H_2O$	1.228 gm. $Na_2HPO_4 7H_2O$
8.0	0.055 gm. "	1.905 gm. "
9.0	0.033 gm. Na_2CO_3	0.290 gm. $NaHCO_3$
10.0	0.218 gm. Na_2CO_3	0.140 gm. $NaHCO_3$

The conidia were examined at intervals of three hours to mark the progress of germination. Approximately one hundred and twenty conidia per treatment were counted. The average length of germ tube as well as the percentage of conidia that germinated were noted.

Experimental Results

Mycelial growth

The growth of C. graminicolum at the various pH levels is illustrated graphically in Figs. 13 and 14. Fig. 13 shows the growth in buffered liquid medium while the growth on unbuffered solid medium is shown in Fig. 14.

The maximum dry weights occur in the region of pH 7.5. Similar results were obtained on solid agar. Some of the buffering materials used appear to have a considerable effect on the growth of the fungus. The borate buffer had the most varied effect on growth. The varied influences of the buffers on growth result in the dis-jointed appearance of the graph (Fig. 13)

Determinations of pH of the buffered media were made after growth. The initial pH values and those after growth are as follows:

before growth	after growth
1.35	1.45
2.10	2.05
2.80	2.80
4.00	4.00
4.95	4.85
5.90	5.90
6.05	5.92
7.00	6.82
6.95	6.75
7.60	7.35
7.30	5.80
8.50	7.85

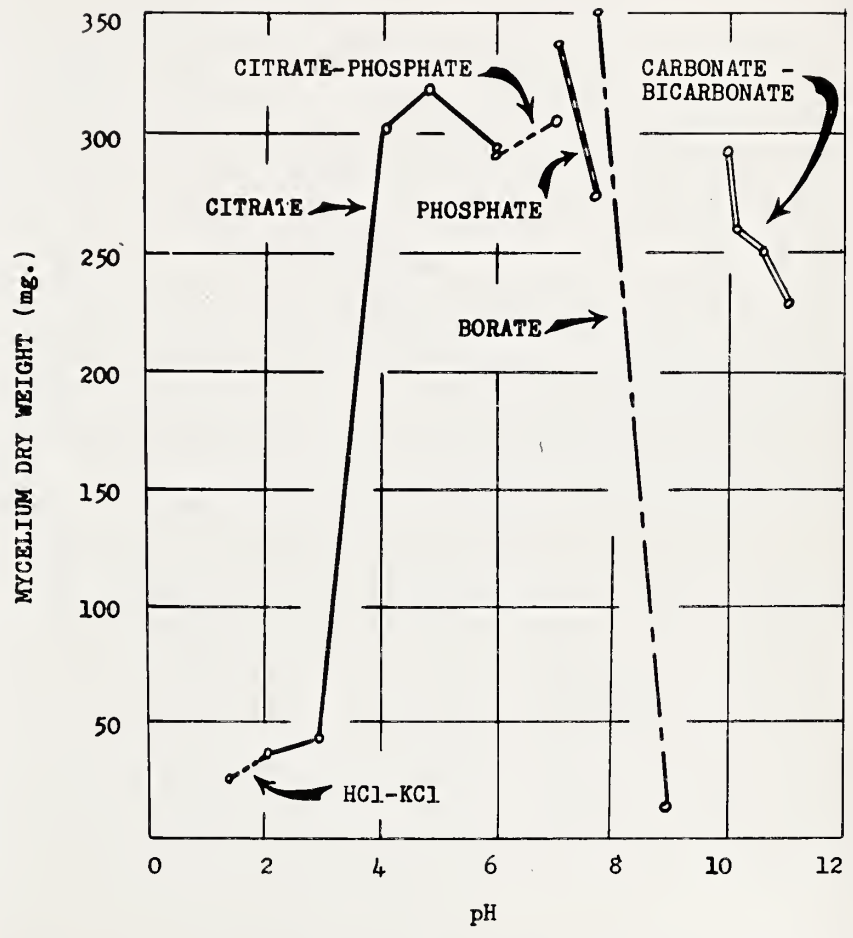

Fig. 13. Dry weights of mycelium of Colletotrichum graminicolum grown in liquid synthetic medium buffered at different pH levels. The buffer systems used are indicated by the arrows.

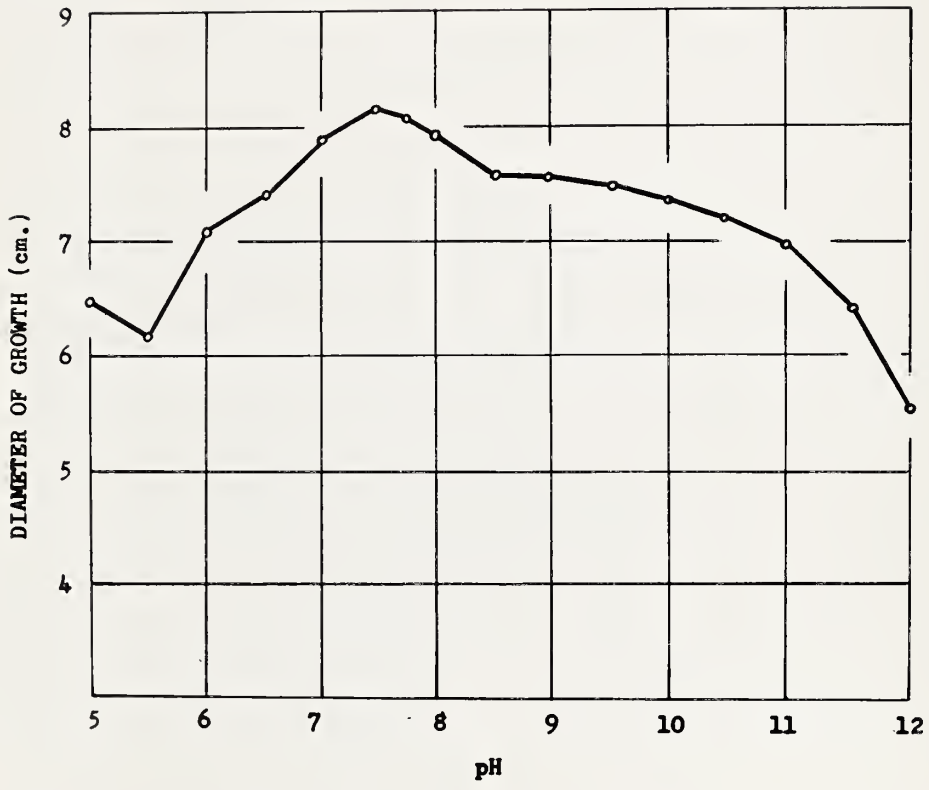

Fig. 14. Diameter of growth of Colletotrichum graminicolum on solid synthetic medium at various pH levels.

before growth	after growth
9.70	7.85
9.80	8.10
9.95	8.40
10.15	8.60

The desired reactions of the media were effectively maintained by the buffers throughout the period of growth except at the high pH values.

Sporulation

The effects of hydrogen-ion concentration on sporulation are shown graphically in Fig. 15. The numbers of conidia produced are shown along with the amount of mycelial growth which occurred at the same pH values. The mycelial growth was rated on the basis of visual observation.

There are two pH optima for sporulation, those being at 5.8 and 8.5. The least sporulation occurred at pH 6.5. The latter pH value resulted in the greatest mycelial growth. The high pH value of 8.5 resulted in the greatest sporulation.

The pH values of the soils were not appreciably changed after growth of the fungus.

Germination

The effects of hydrogen-ion concentration on germination of conidia of C. graminicolum are shown graphically in Figs. 16 and 17.

Two pH optima for germination were detected. The optima were at pH 5 and pH 8. These results were obtained for both percentage of

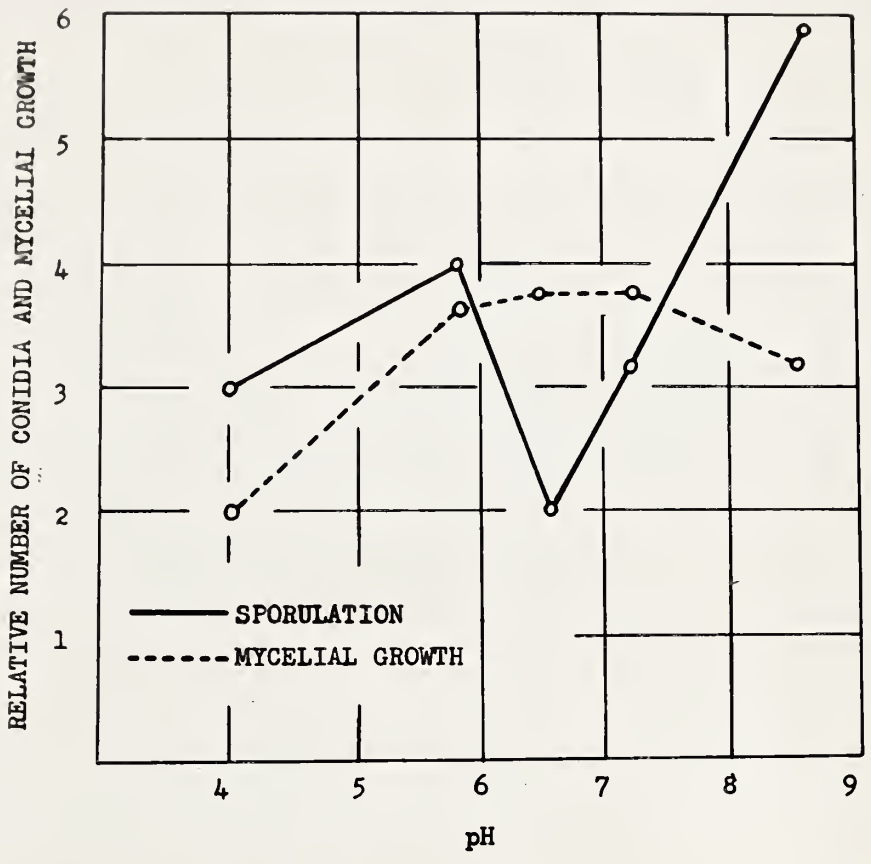

Fig. 15. Amount of growth of mycelium and production of conidia by Colletotrichum graminicolum on a soil-cornmeal medium at various pH levels.

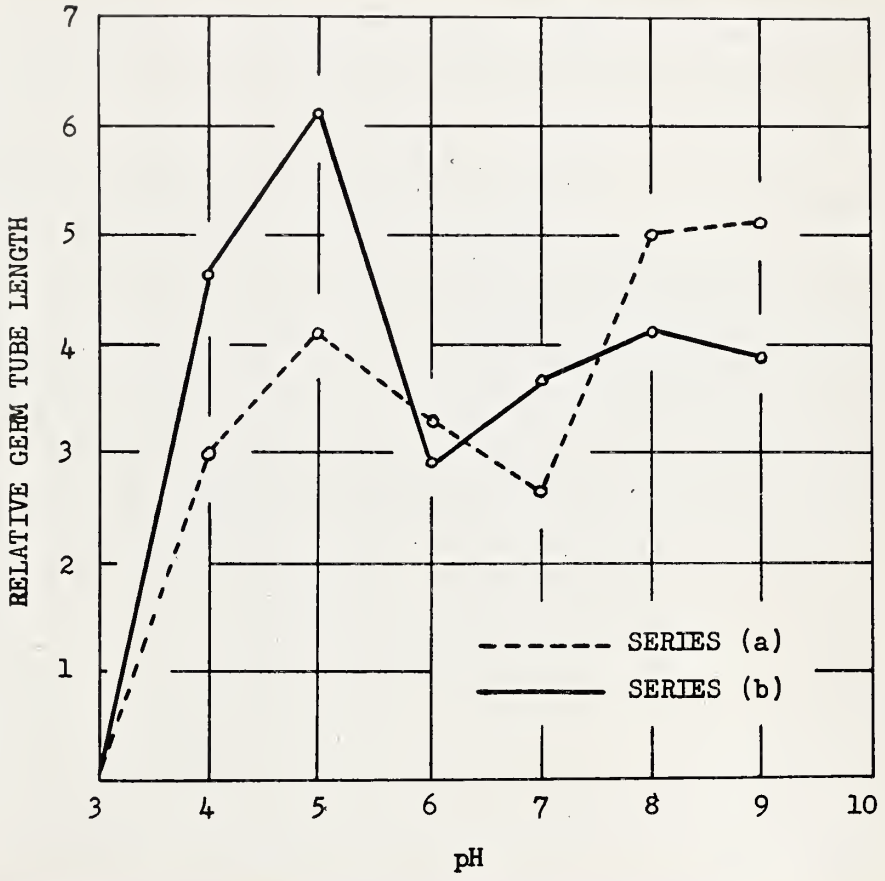

Fig. 16. Length of germ tubes of conidia of Colletotrichum graminicolum at different pH levels. Series (a) and (b) show germ tube elongation on two parallel buffer systems.

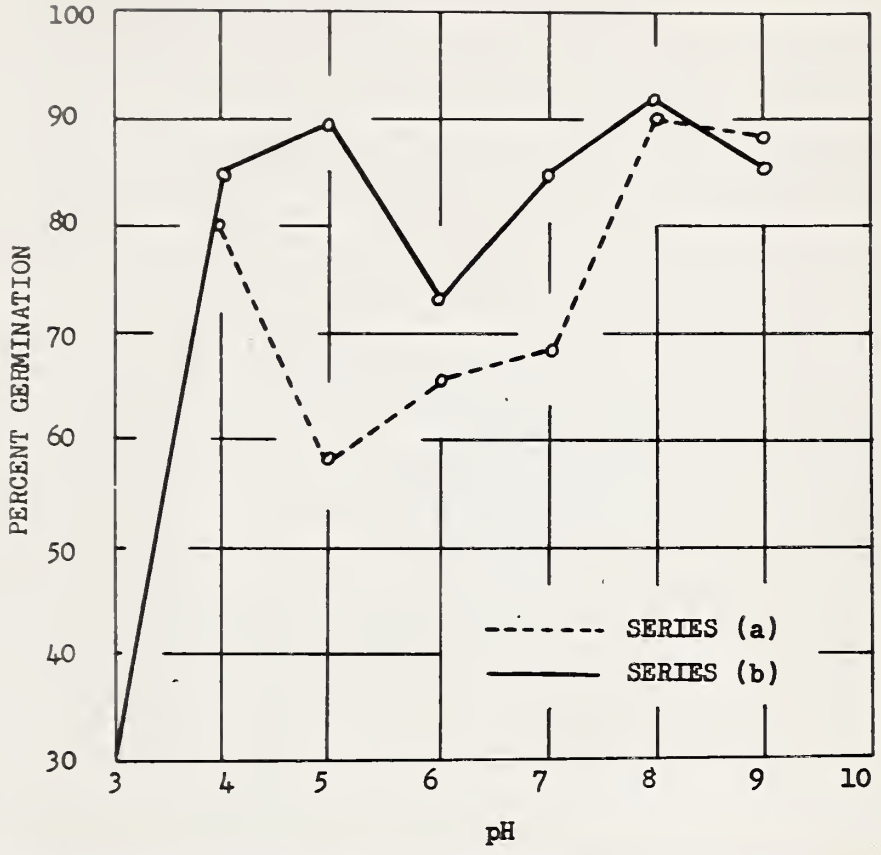

Fig. 17. Percentage of conidia of Colletotrichum graminicolum germinating at different pH levels. Series (a) and (b) indicate the percentage germination on two par allel buffer systems.

germination of conidia and growth of the germ tube.

Discussion

Colletotrichum graminicolum responds favorably to an alkaline reaction. A wide range of pH is tolerated. The fungus shows more deviation from the "normal" than the majority of fungi, as the pH optima for the various physiological activities are at the upper limits of pH optima for fungi. There is little significance of the pH optima in the present investigation.

FUNGISTATIC EFFECTS OF SOIL

It was previously stated that there was a possible relationship between the severity of anthracnose and soil conditions. This relationship could possibly be explained on the basis of the fungistatic effects of the soils on Colletotrichum graminicolum. Investigation of the fungistatic effects of soils on C. graminicolum involved: the demonstration of a fungistatic effect, a study of what the fungistatic factor(s) might be, and a determination of the relative sensitivity of C. graminicolum to antibiotic producing organisms.

Literature Review

Dobbs and Hinson (13) reported widespread fungistatic effects of numerous soils. Inhibition was invariably complete or nearly complete. Their findings have been confirmed by other workers (23, 32, 37) for a large number of soils.

Numerous attempts have been made to determine the nature of the inhibition. Lack of nutrients, moisture levels, levels of oxygen or other gases, inorganic toxicants, and complex organic substances such as antibiotics have been considered.

Dobbs, Hinson, and Bywater (14) reported that soils are variable in their inhibitory effect. Some soils inhibited germination of spores of Mucor ramannianus under all circumstances, in summer and winter, after washing, freezing, and after autoclaving with or without the addition of glucose. However, the majority of soils when heated lost all inhibiting power and usually stimulated growth and sporulation. Lockwood and

Lingappa (33) inoculated sterilized loam soil with randomly selected isolates of soil actinomycetes, fungi, and bacteria. After incubation for one week the soil inhibited germination of conidia of Glomerella cingulata. Mycelium of G. cingulata on agar was lysed by fourteen of the twenty actinomycetes as well as by soil inoculated with the same fourteen isolates. Two of the twenty-seven bacteria lysed mycelium on agar, but none did in soil. None of the fungi lysed living G. cingulata mycelium. Most of the actinomycetes producing an inhibition zone on seeded agar also lysed living G. cingulata mycelium. This relationship did not hold for inhibitory fungi or bacteria. Similar results were obtained by Lockwood (32) when he found that soils which had lost their power of inhibition through sterilization with steam or propylene oxide regained their inhibitory properties when inoculated with mycolytic isolates of Streptomyces sp. The lytic and inhibitory effects were similar to those produced on natural (unsterilized) soil. Jackson (23) found a decreasing fungistatic effect with increasing soil acidity. It was not known whether the effect was due to the inactivation of some basic compound or to the reduction in numbers of antibiotic-producing organisms. Griffin(19) showed that organisms need not be antagonistic in culture or produce a specific antibiotic substance in order to produce a fungistatic effect in soil. Dobbs and Hinson (13) showed that the addition of glucose or plant residues to the soil often overcomes the inhibitory effects.

It is generally agreed that inhibition in soils is of biological origin. Most of the factors such as nutritional deficiencies or inorganic toxicants have been systematically eliminated. However, the mechanism of inhibition is not yet clear. The soil population is complex, and it is

difficult to determine which components are involved.

The production of antibiotics appears to offer the best single explanation for all of the phenomena observed. However, Brian (7) considers it improbable that antibiotics are produced naturally in soils. Lingappa and Lockwood (30) also could not demonstrate the accumulation of antibiotics in the soil, but they still considered the evidence to favor the production of antibiotics. There is evidence that antibiotics are produced on fresh organic substrates in micro-environments of the soil. Lingappa and Lockweed (30) suggested the possibility of nutrients diffusing from the fungus spores and stimulating surrounding microflora. The result was inhibition of germination of fungus spores. There is much evidence that the growth of soil microbes on or in the vicinity of fungal structures is involved in the over-all fungi-toxicity.

Pridham, et al (37) surveyed five hundred different species of Streptomyces for effects on plant pathogens, both fungal and bacterial. Products from the growth of some of the Streptomyces species showed activity against plant diseases. A mixture of antibiotics produced by a strain of Streptomyces cinnamomeus was found to be effective against rust, anthracnose, downy mildew and powdery mildew of beans, stem rust of wheat, and powdery mildew of blue-grass. It is of interest to note that the anthracnose fungus on snap beans, Colletotrichum lindemuthianum, was especially sensitive to the antibiotics.

Methods And Materials

Determination of soil fungistasis

The method used in this investigation was the direct assay of

Lingappa and Lockwood (31), with some modifications. Moistened soil was placed in a Petri dish, and compacted to a smooth hard surface. A conidial suspension of C. graminicolum was sprayed on the compacted soil surface. After a period of incubation the conidia were recovered by pressing a one mm. thick strip of 1.5 percent water agar on the soil surface. The agar was removed, and the contact surface was stained with cotton blue in lacto-phenol, and examined for germination of conidia. Black loam, peat-soil, Grey Wooded, Brown loam, sandy Brown, and Brownclay were the types of soil used. The test was performed on natural and autoclaved soils.

Numbers of antagonistic organisms in various soils

The three-layer plate method of Herr (22) was employed. It involved pouring a base layer consisting of 10 ml. of two percent water agar into sterile Petri dishes, which was followed by 1 ml. of a soil dilution in 5 ml. of 1.5 percent water agar. After two days of incubation at 24°C, the final layer, a 2 ml. suspension of C. graminicolum in 3 ml. of 2 percent Czapeck's agar was poured. Zones of inhibition were noted after six days. Twenty two different Alberta soils were tested. Each soil was diluted 1:25,000 with distilled water. Each treatment was replicated six times.

Eleven organisms antagonistic to C. graminicolum were isolated from the soil dilution plates. Ten of these organisms, designated as S1 to S10 were Streptomyces species, and the remaining one, designated as B1, was an unknown bacterial antagonist.

Relative sensitivity of C. graminicolum to antagonists

Agar-plate assays were used to determine the sensitivity of C. graminicolum to the various microbial antagonists. Potato-sucrose agar was dispensed into Petri dishes. One side of the agar in the Petri dish was inoculated with an antagonistic organism, and the opposite side was inoculated with the test fungus. After a period of incubation the zone of inhibition was measured. The sensitivity of C. graminicolum was compared to that of Rhizoctonia solani and Fusarium culmorum. Each treatment was replicated six times.

In vivo determination of fungistasis

It was desirable to determine whether the antagonisms produced on agar plates would also occur in the soil. A nylon mesh technique as described by Old and Nicolson (36), with some modifications, was employed. Natural saran, with 400 openings per square centimetre was substituted for nylon mesh. The saran was obtained from the Lumite Division, Chicopee Manufacturing Corp., Cornelia, Georgia. The saran mesh was chemically inert, and could be autoclaved without damage.

Autoclaved strips of the saran mesh, 1 cm. x 5 cm., were dipped into melted potato-sucrose agar, removed, and the agar allowed to harden. The film of agar in the mesh served as a substrate for the growth of C. graminicolum. Discs of C. graminicolum were placed on the centre of the strips, and incubated for two days. The strips with the slight growth of fungus were then placed in soil which had been previously sterilized, inoculated with an antagonistic organism, and incubated for one week. The fungus on the strip was incubated for one week, after which it

was removed, brushed lightly to remove soil particles, and examined under 100x magnification.

The extent of the growth of C. graminicolum was measured by the numbers of squares of the mesh over which the fungus extended.

The effects of four of the Streptomyces species and the bacterial antagonist were tested in this manner. A control of non-inoculated soil was included. Each treatment was replicated four times.

Effects of water extracts

A test was made to determine the effects of hot water extracts of a number of different soils on the growth of C. graminicolum. Soil was stirred in boiling water, in the ratio of 2:1 (vw), for ten minutes. It was then vacuum-filtered. The soil residue was rinsed with a further 300 ml. of hot water. Approximately three litres of each soil extract were obtained. Two litre aliquots of each extract were concentrated to one-half volume. This resulted in "dilute" and "concentrated" extracts of each soil. Seven Alberta soils, three of which were Grey Wooded, one peat, two Brown, and one Black, were treated in this way. The "basal" synthetic medium (with 0.15 gm. ammonium nitrate per litre) was used to determine the effects of the extracts on the growth of C. graminicolum. Soil extracts were substituted for de-mineralized water. A control with water was included. The amount of sucrose was reduced from 38 grams per litre to 25 grams per litre. The procedure for shake culture and mycelial dry weight determinations, as previously described, was employed. Growths greater or less than the water control were con-

sidered as stimulation or inhibition respectively.

Experimental Results

Direct assay for spore germination

Germination of conidia of C. graminicolum was completely inhibited in natural but not in autoclaved soils. Fig. 18 shows conidia recovered from natural soil. The conidia in the photograph are the crescent-shaped bodies interspersed among the soil particles. Fig. 19 shows germ tubes arising from conidia of C. graminicolum in autoclaved soil. Arrows indicate the location of the germ tubes.

Numbers of antagonistic organisms in various soils

All of the soils contained some organisms antagonistic to C. graminicolum. The results are summarized in Fig. 20. They are given as the number of antagonistic organisms per dilution plate. Fig. 21 shows zones of inhibition produced by the antagonistic organisms.

Soils with higher organic matter levels generally contained more antagonistic organisms than did the degraded soils. The peat soils contained especially high numbers of antagonists. The latter antagonists also produced larger zones of inhibition than antagonistic organisms from degraded soils.

The soils used in these determinations were collected during survey trips, and so disease ratings are included in Fig. 20. There was no correlation between disease severity and the numbers of antagonistic organisms in the soil.

Fig. 18. Non-germinated conidia of Colletotrichum graminicolum recovered from natural (unsterilized) soil. The conidia are the crescent-shaped bodies interspersed among the large black soil particles. X100.

Fig. 19. Germ tubes of conidia of Colletotrichum graminicolum which have germinated in autoclaved soil. Arrows mark the location of the germ tubes. X100.

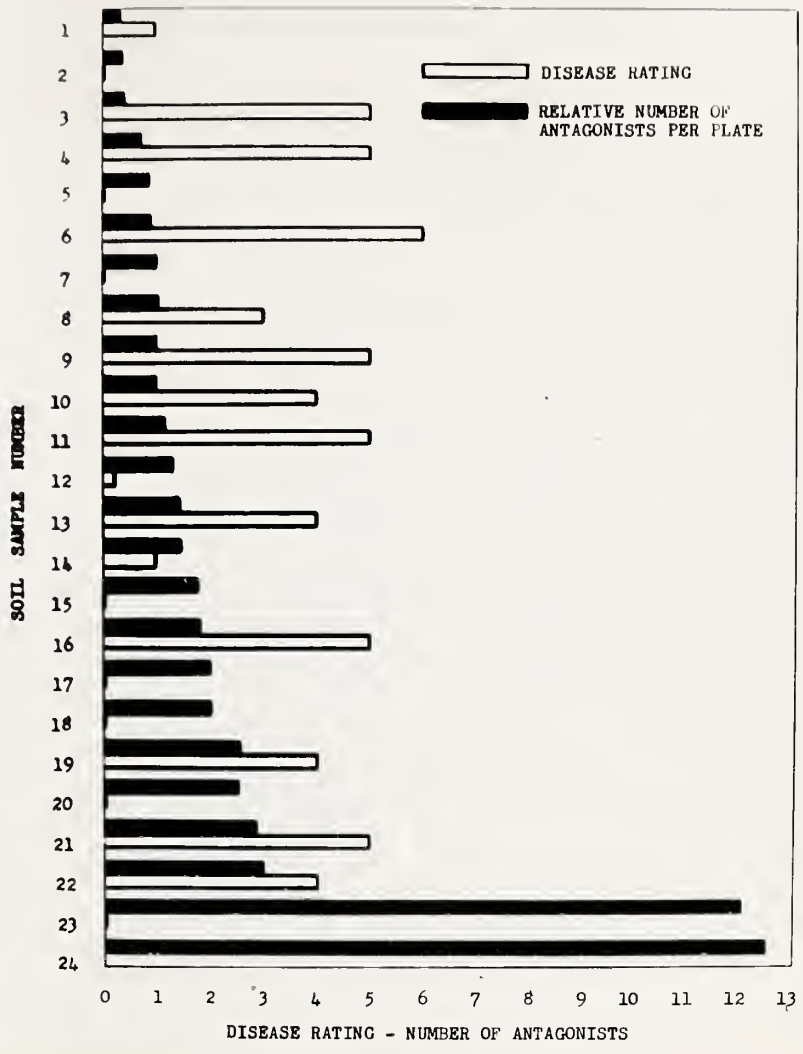

Fig. 20. Relationship between the numbers of antagonistic soil organisms and the severity of cereal anthracnose.

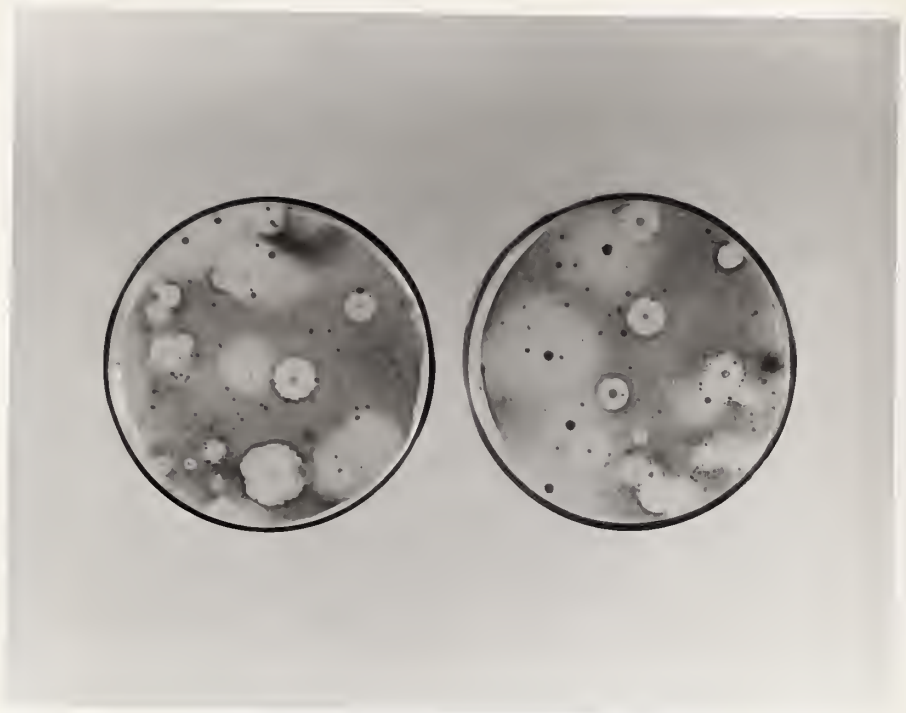

Fig. 21. Zones of inhibition of Colletotrichum graminicolum produced by antagonistic soil microorganisms. The zones of inhibition are the clear areas in the dark background. The antagonistic organisms are the colonies at the centre of the clear areas.

Relative sensitivity of C. graminicolum to antagonists

The degrees of inhibition by the Streptomyces spp. to C. graminicolum, R. solani, and F. culmorum are shown in Fig 22. Both strong and weak inhibitions exist against all three fungi. However, in all cases, the inhibition to C. graminicolum was greater than against the other two fungi. F. culmorum and R. solani are inhibited to approximately the same extent.

Fig 23 shows the degrees of inhibition of four different Streptomyces spp. to C. graminicolum. Fig. 24 shows the degrees of inhibition of one Streptomyces spp. to each of C. graminicolum, R. solani, and F. culmorum.

In vivo effects of fungistasis

The unknown bacterial isolate was the only antagonist that failed to inhibit the growth of C. graminicolum in the soil, although it showed inhibition on agar cultures. Inhibition by the Streptomyces spp. in the soil appeared to be similar to that in agar cultures. The results are shown graphically in Fig. 25. Inhibition by the Streptomyces spp. was nearly complete.

The extension of growth of C. graminicolum in the soil cultures is shown in Fig. 26 (a - c). Mycelial strands are shown between the squares of the saran mesh.

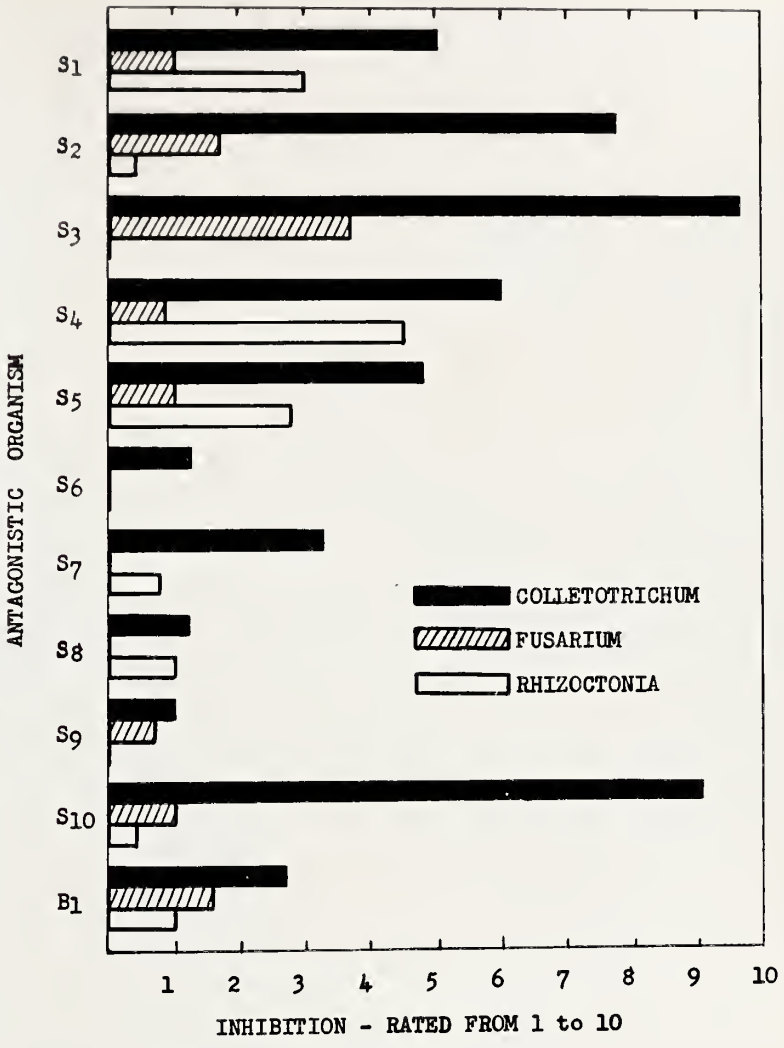

Fig. 22. Comparison of inhibition of Colletotrichum graminicolum, Rhizoctonia solani, and Fusarium culmorum by eleven different antagonistic soil organisms.

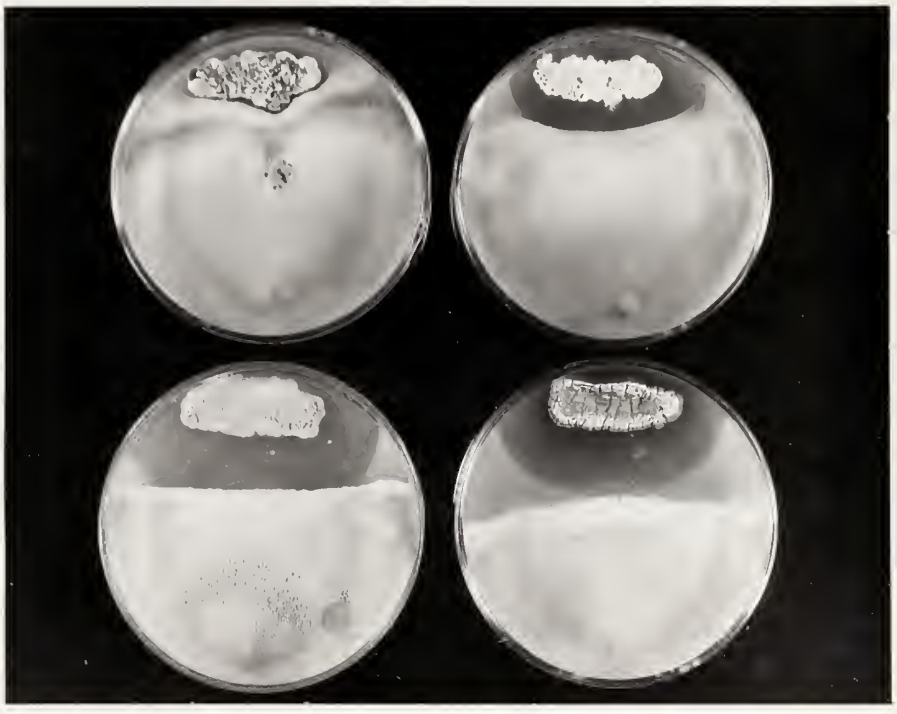

Fig. 23. Inhibition of Colletotrichum graminicolum by four Streptomyces spp. Top left, S_9; top right, S_5; bottom left, S_2; bottom right, S_3.

Fig. 24. Inhibition of Colletotrichum graminicolum, Rhizoctonia solani, and Fusarium culmorum by one Streptomyces spp. Top left, C. graminicolum; top right, R. solani; bottom, F. culmorum.

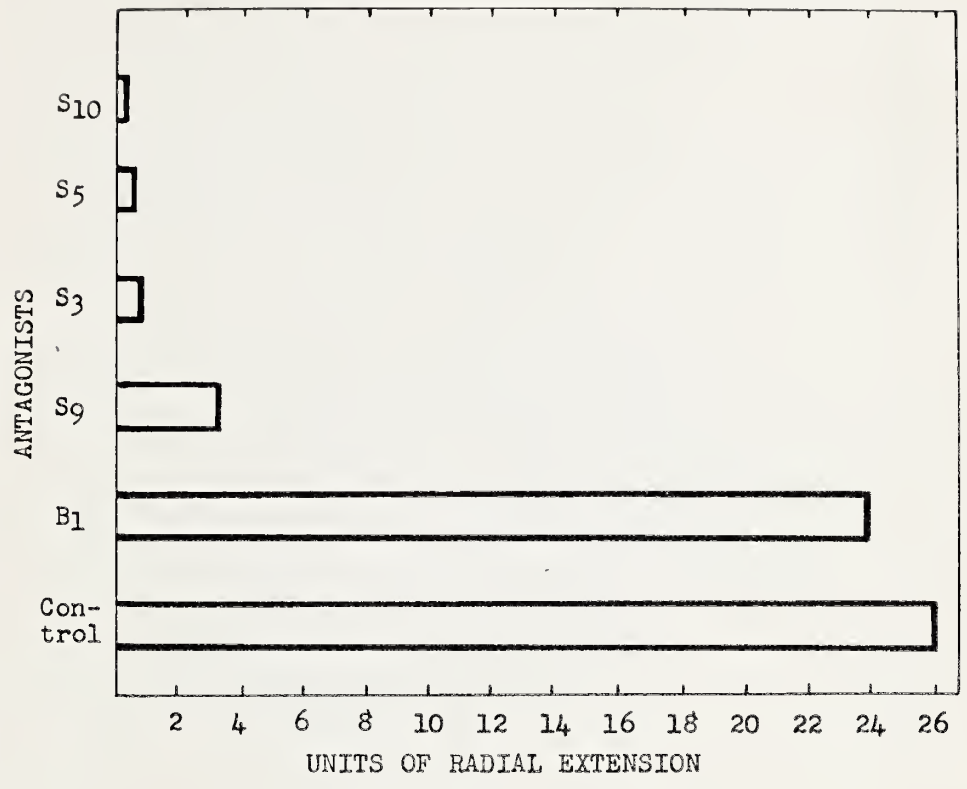

Fig. 25. Extent of inhibition of Colletotrichum graminicolum by antagonistic organisms in soil as measured by the saran mesh method.

(a)

(b)

(c)

Fig. 26. Extent of growth of Colletotrichum graminicolum in soil inoculated with antagonistic soil organisms as determined by the saran mesh method. (a) control, (b) moderate inhibition by a weak antagonist, and (c) complete inhibition. X100.

Growth in aqueous soil extracts.

The results of growth of C. graminicolum in aqueous soil extracts are shown in Fig. 27. The centre line designated as control represents the growth of the fungus in synthetic medium made up with water. The growth of the fungus in synthetic medium made up with the extracts from each of the seven different soils is shown as percent inhibition or stimulation. Soil extracts (a) and (b) were inhibitory, and extracts (e), (f), and (g) were stimulatory. Soils (c) and (d) were practically neutral in their effect.

There was no correlation between disease incidence and the effect of the extracts on the growth of C. graminicolum.

Discussion

The soils that were investigated exhibited inhibitory effects on C. graminicolum. Spore germination on the natural soil was completely inhibited as demonstrated by the direct assay while autoclaved soils did not show this effect. These results are in general agreement with those obtained by other workers studying soil fungistasis. The direct assay for spore germination appears to be a particularly suitable technique because it simulates closely natural conditions.

The nature of the fungistatic effect is more difficult to explain. The differences in effect of natural and autoclaved soils suggest that the effect is biological. This is in agreement with the opinions of most workers in soil fungistasis. Any possible differences in the effect of different soils could not be detected by the direct

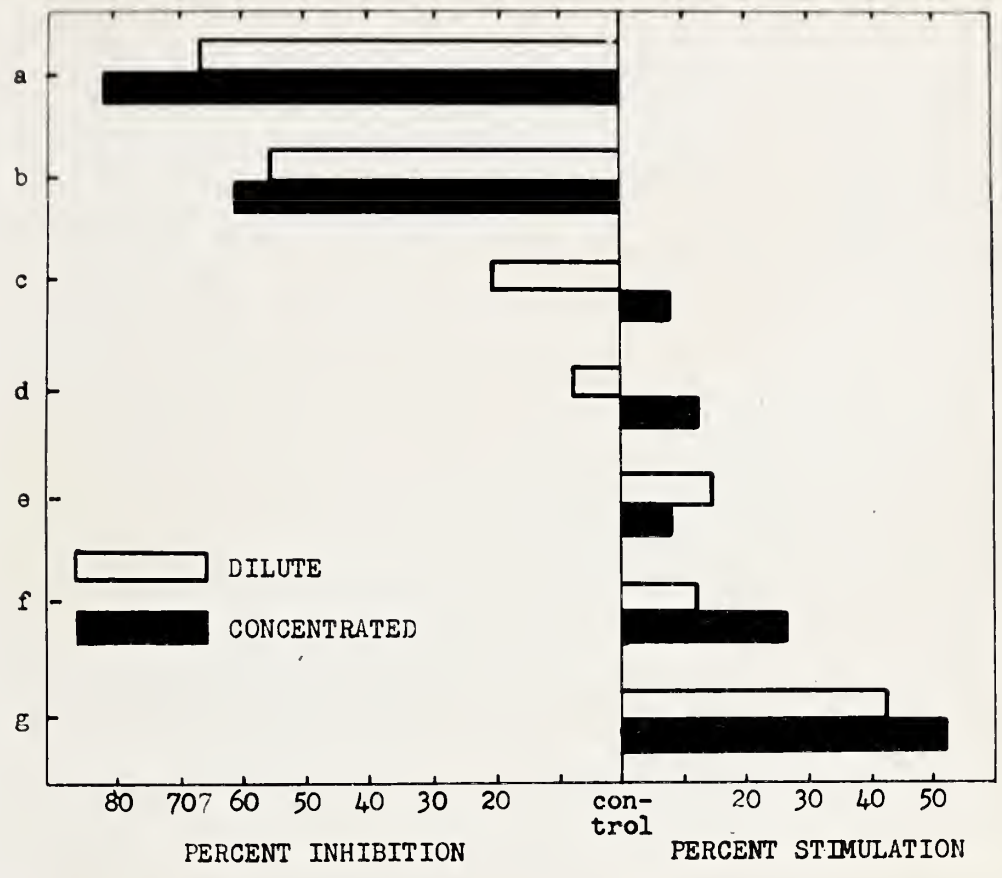

Fig. 27. Effects of aqueous, autoclaved soil extracts on the growth of Colletotrichum graminicolum. The effects of dilute and concentrated extracts of seven representative Alberta soils are shown.

assay. The fungistatic effects could possibly be determined by the relative numbers of antagonistic organisms in the various soils. Significant differences in the numbers of antagonistic organisms were detected. The degraded type soils generally had fewer antagonists, while those soils with high organic matter levels contained higher numbers of antagonists. The peat soils contained nearly ten times the number of antagonistic organisms as compared to the less organic soils. The increased inhibitory effects of peat soils are well known.

An attempt was made to correlate the numbers of antagonists with disease incidence. There was no correlation. However, these results may have been influenced by the existence of physiologic races of the fungus and crop history. The antagonism of certain organisms as shown in agar and soil cultures may be an important factor that influences the severity of cereal anthracnose. Significant effects, however, are not evident unless the numbers of antagonistic organisms are very high, as in the peat soils.

The fact that the antagonisms that occur on agar plates can occur in the soil was confirmed by the "in vivo" tests. Effects of the antagonists in soil were similar to those produced on agar plates. The bacterial antagonist did not show as strong inhibition in soil as on agar plates. This corresponds to the results of Lockwood and Lingappa (33), who also found the results of bacterial antagonists in soil to differ from those on agar. It appears as though Streptomyces spp. play an important role in the inhibition of C. graminicolum by soil.

Colletotrichum graminicolum is more sensitive to the antagonistic

organisms than are either Fusarium culmorum or Rhizoctonia solani. These antagonists were selectively chosen from Colletotrichum-seeded plates; but the fact that all eleven antagonists have greater effects on C. graminicolum than on the other two fungi suggests that the fungus is particularly sensitive to antibiotic-producing organisms. Garrett (17) has suggested that those fungi which are more sensitive to antibiotics are generally poor competitors in the soil. Results of these experiments indicate that C. graminicolum is not a strongly competitive organism in the soil. Further implications of this will be discussed later.

The growth of C. graminicolum in soil extracts suggests that fungistasis is not wholly biological. Biological inhibitors in the soil are generally found to be heat-labile. The autoclaving of the extracts thus would destroy any of these which are present. This is evident from the results of germination on sterilized and unsterilized soil. However, several of the soil extracts strongly inhibited growth. The inhibitory substanceswere not identified. It is of interest to note that three of the soils, one of which had inhibitory effects, one had little effect, and the remaining was stimulatory, were all typical Grey Wooded soils. This, then, suggests that the non-biological inhibition is not related to the type of soil. A further observation is that the peat soils, which contained high numbers of antagonists, stimulated growth of C. graminicolum when their extracts were used. This is most likely a nutritional response. However, no anthracnose has yet been found on crops on peat soils.

In summary, these experiments show that soils in Alberta have strong inhibitory effects on C. graminicolum, and that the inhibition may be both biological and non-biological. High numbers of antagonistic organisms in the soil probably limit the development of cereal anthracnose.

GENERAL DISCUSSION AND CONCLUSIONS

The main objective in this investigation was to determine the factors which influence the severity of cereal anthracnose. The results of the experiments will be discussed in relation to the occurrence of anthracnose as determined by field observations.

Anthracnose of cereal crops in Alberta is most severe in north-central Alberta, and is practically non-existent in the brown soil regions. Disease incidence is often higher on degraded type soils within regions where the disease is found. Anthracnose does not occur on crops grown on soils which have a very high organic matter content. It should be emphasized that these observations are based on a one year survey. Climatic conditions change from year to year, and this is likely to influence the incidence of anthracnose. However, the following discussion will be based on general conditions as they occur in Alberta.

The Brown soil zones are characterized by low annual precipitation, frequent drought, high evaporation, and frequent hot, dry winds. These soils are relatively low in nitrogen, phosphorus, and organic matter. They are often saline. In the Black soil zones precipitation is higher, evaporation is less, and nitrogen and organic matter contents are about three to four times that of average Brown soils. In Grey Wooded soil zones precipitation is similar to that of Black soils, but temperatures are generally cooler, evaporation is lower, and the growing season is shorter. The Grey Wooded soils are usually deficient in nitrogen, organic matter, and phosphorus. These soils are generally less fertile

because of leaching. These are the major divisions of soil and climatic conditions within the portions of Alberta which were covered by the survey for anthracnose. These conditions will be related to the results of the investigations carried out.

The growth of Colletotrichum graminicolum is favored by low nitrogen and high temperature. Disease development is also greater at high temperatures. Although no correlation between disease development and pH was established, Ali (1) found the percentage of seedling blight was greatest in soils at pH values of 7.0 to 9.0. Investigations showed C. graminicolum to be favored by relatively high pH. Low organic matter content of the soil appears to favor disease development.

High levels of disease incidence would be expected on the Brown soils of the south on the basis of the above information. The conditions there closely correspond to the conditions which favor the pathogen as well as disease development. However, field observations indicate that the opposite situation exists. The influence of moisture was not strongly considered as a good portion of the Brown soils are under irrigation. The anthracnose fungus was found as a saprophyte in some fields of one-year old stubble near Lethbridge, and so it can be concluded that the fungus is present in southern areas. Factors such as cooling due to evaporation, crop rotation, influence of relative humidity, salt concentration in the soil, and other possible factors need to be considered. In this respect Ali (1) found that concentrations of potassium nitrate, ammonium nitrate, and magnesium sulfate in the soil greatly influenced disease development. Correlation between mineral concentration as determined

by the soil analysis and disease severity did not occur. However, this may not be true under more closely controlled conditions. The possibility that host specialization exists may cause the effect of crop rotation to obscure influences due to other factors.

In northern areas where anthracnose is plentiful, some trends are more evident. High organic levels in the soil reduce disease incidence. This can best be explained on the basis of the ability of C. graminicolum to survive in the soil. A high organic matter level in the soil stimulates the growth of many soil organisms, especially Streptomyces spp. Species of Streptomyces antagonistic to C. graminicolum were more plentiful in soils with high organic matter content. Also, C. graminicolum is more sensitive to the antagonistic organisms than are some other common soil fungi. Garrett (17) has indicated that the sensitivity of a soil fungus to antibiotics produced in the soil is an indication of the survival ability of that fungus. Present indications are that C. graminicolum is a weak competitor in the soil. This, at present, appears to be the reason for the reduced incidence of anthracnose on crops grown in soils which have high levels of organic matter. A similar situation may exist in the degraded soils, where nitrogen and organic matter is low. In this situation C. graminicolum would be better able to compete because of its lower nitrogen requirements and because of a reduced number of antagonists. This could result in a higher inoculum potential for the fungus, hence higher disease incidence. The competitive saprophytic abilities of a number of fungi have been related to the occurrence of the diseases which they cause (17).

Conidia are usually prime agents of infection. Temperature appears to have the greatest influence on the numbers of conidia produced. The optimum temperature for sporulation by C. graminicolum is relatively lower than that for mycelial growth. Therefore, early season cool temperatures could greatly increase the numbers of conidia, resulting in greater numbers of infection courts. This may partially account for the higher incidence of anthracnose in the northern regions of Alberta.

No single factor appears to be responsible for higher or lower levels of cereal anthracnose. The effects of temperature, low nitrogen requirements, and the ability of C. graminicolum to survive in the soil appear to be significant features. However, further studies, which would be helpful in explaining the distribution of cereal anthracnose in Alberta are needed. These should particularly include other nutritional requirements of the pathogen, and the extent to which host specialization occurs. Surveys should be conducted for the next few years in order to determine more accurately the influence of variations in weather on the incidence of this disease throughout Alberta.

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